Bio innovation

07

Versatile Luciferases
New Tools for Reporter Assays

Improving Productivity
Through Compact Automation

MSIA™ Model system
A Universal Mass Spectrometric Immunoassay

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Welcome

I am delighted to welcome you to our 7th edition of the Bio-Innovation series, an educational publication focused on new and emerging trends for every life scientist in Australia and New Zealand.This edition of Bio-Innovation focuses on the most recent technology platforms and products released to market by Thermo Fisher Scientific.

Thermo Fisher Scientific recognises that the local landscape for scientists is becoming more challenging and more competitive from a funding perspective. By investing in our life science community with publications such as this we hope to assist you to increase efficiency and give you the freedom to focus on your research. Our goal is to help you succeed. However you measure success, we develop the technology platforms; provide the application resources and the programs to help you achieve it.

Our organisation is built on over a century of experience, both locally and globally. We are over 750 people in Australia and New Zealand working together to deliver innovation and create connections across our portfolio to accelerate customer results and serve science in ways no one else can.

One of the great things about our organisation is our mission. We enable our customers to make the world healthier, cleaner and safer. I see this in practice often as I travel to our customers' laboratories and see our technology platforms, from instruments to consumables helping researchers identify biomarkers of disease, so they can develop better treatments and one day find a cure.

I would like to take this opportunity to thank you for choosing Thermo Fisher Scientific. I hope this edition of Bio-Innovation provides you with some useful insight and assists you to achieve your results. I look forward to hearing directly from you and hope to meet you during my travels.

Tony Acciarito
Director, Scientific Business
Thermo Fisher Scientific

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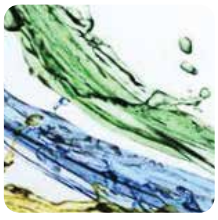

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Nucleic acid isolation being one of the most fundamental steps in molecular biology applications is a prerequisite for successful experimental workflow. MagJET nucleic acid purification kits utilise magnetic bead-based technology allowing for highly efficient nucleic acid isolation from a variety of samples at any throughput. MagJET kits provide high-purity DNA and RNA ready to use in routine and demanding downstream applications.

MagJET DNA & RNA purification kits

The proprietary MagJET micro beads

MagJET magnetic bead-based technology utilises proprietary high capacity paramagnetic particles optimised to isolate nucleic acids with superior purity and yields compared to other kits on the market.

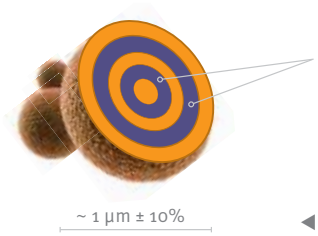
Due to large total surface area, MagJET micro beads exhibit high binding capacity, resulting in superior nucleic acid yields and recovery rates typically exceeding 80%. Uniform bead size and shape ensure robust performance and consistent results with less sample-to-sample variation.

The MagJET micro beads are highly stable in solution and respond rapidly to an applied magnetic field making the MagJET kits an ideal choice for high-throughput automation.

Designed for great performance

Magnetite content ~60%

- High binding capacity • Fast magnetic response
- Uniform size and shape • Ideal for automation
- High recovery (>80%) • Proprietary technology



Automated and manual workflows

MagJET nucleic acid isolation technology is suitable both for high throughput automated and manual sample processing.

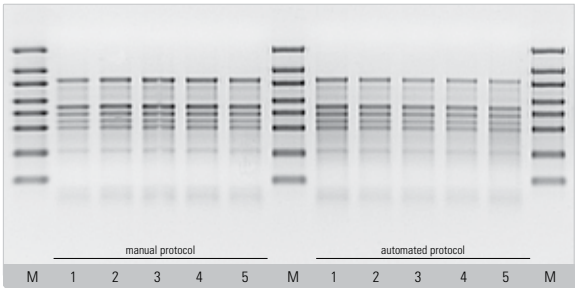
(A) The automation protocols are optimised for Thermo Scientific KingFisher instruments where magnetic beads are transferred from well to well during the purification procedure.

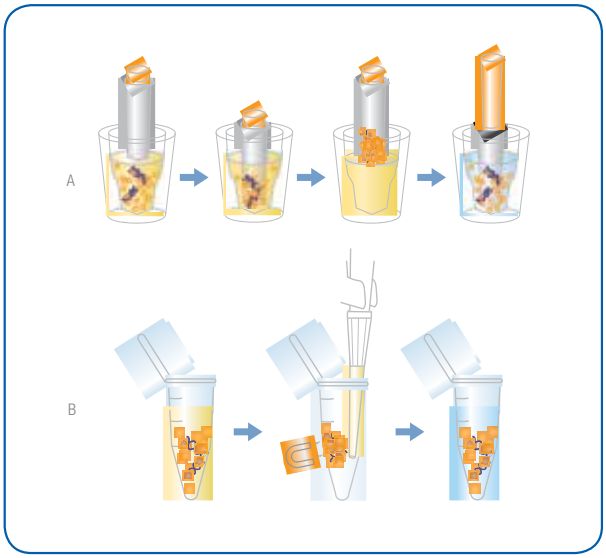
(B) In the manual protocol a magnetic field applied by an external source is used to capture the beads against the wall of the tube. The supernatant is removed by aspiration.

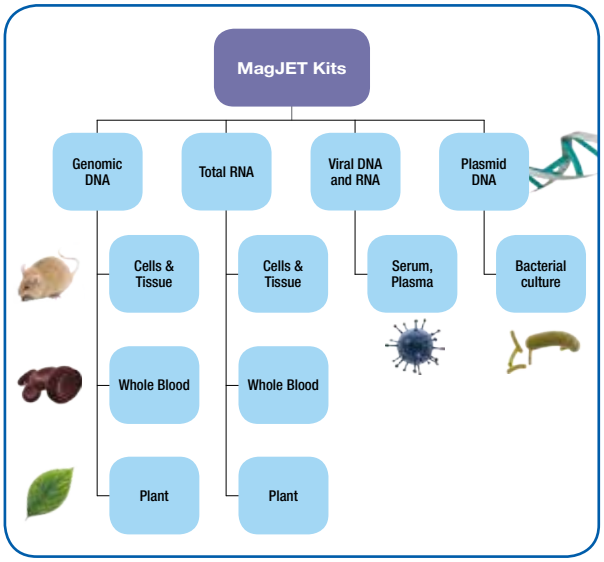
▸ get attracted to great results!

Robust Performance

Manual and automated workflows were used to purify RNA from *N. tabacum* leaf samples (each 50 mg) with the MagJET Plant RNA Kit. The Thermo Scientific™ KingFisher™ protocol was used for the automated workflow. Isolated RNA was analysed by agarose gel electrophoresis (1%), M – Thermo Scientific™ RiboRuler™ High Range RNA Ladder (Cat #SM1823).







With pharmaceutical companies investing heavily in extensive R&D to produce effective vaccines for existing and emerging health risks, there is a growing recognition that protecting the potency and viability of a vaccine during storage is essential.

Effective vaccine storage

One of the most rapidly expanding pharmaceutical markets today, the vaccine therapy category protects public health globally and saves an estimated three million lives each year [1]. However, incorrect refrigeration and storage of vaccines can lead to costly mistakes. In clinical practice, refrigeration problems have been cited as a major cause of the $20 million wasted each year from ruined vaccines in the U.S. Federal Vaccines for Children Program [2]. In addition, a recent UK study revealed that 40% of vaccines were kept at the incorrect temperature, which could potentially damage a vaccine's potency and effectiveness [3].Because it is not apparent if a vaccine has become sub-standard through exposure to temperature fluctuations, incorrect storage temperatures can lead to a very real danger that patients are given a sub-standard vaccine, leaving them unprotected against dangerous diseases. Storing vaccines in controlled and constant temperature conditions is therefore the key to protecting a population at risk. Correct vaccine refrigeration also reduces expensive wastage of high-value and fragile biological samples, eliminating the need for costly revaccination programmes that may have to be initiated if the integrity of a vaccine becomes compromised.

Choosing the correct storage

There are significant differences in levels of performance and reliability across various categories of cold storage equipment. Critical cold storage applications demand a safe, stable environment; the temperature fluctuations found in household or commercial cabinets must be eliminated. Choosing the appropriate equipment with proven performance that meets all government standards is now an important business decision for research, hospital, pharmacy and clinical laboratories. Today's specialised laboratory refrigerators and freezers are well equipped to provide efficient high-value biological sample storage that maintains vaccine integrity. Improvements in design and technology ensure that advanced refrigeration can keep vaccines at the constant conditions that clinics and laboratories require.

Maintaining constant temperature

The majority of commonly administered vaccines need to be stored between 2 and 8 °C and must not be exposed to freezing temperatures, which will irreversibly reduce their titre. However, the development of the varicella vaccine in 1995 and the more recent introduction of the live attenuated influenza vaccine (LAIV) have increased the complexity of vaccine storage.

These vaccines, including MMR, Varicella (Chickenpox) and Zoster (shingles), as well as LAIV must be maintained in a frozen state (-18 °C is recommended) without any freeze-thaw cycles occurring [5, 6]. Temperature fluctuations can result from sample retrieval by multiple users, unpredictable defrosting cycles or poor insulation. Accidental freezing of

freeze-sensitive vaccines, which represent over 31% of the vaccines on which UNICEF spent €318 million in 2005 [6], can also irreparably damage the chemical structure of a vaccine, rendering it ineffective. In addition, certain freeze-sensitive vaccines contain an aluminum adjuvant that precipitates when frozen, resulting in a loss of efficiency and potency. To prevent temperature deviations, modern clinical refrigeration equipment provides microprocessor-controlled in-built monitoring systems. These can include a graphic thermometer that confirms normal, high or low temperature conditions, with the optional ability to automatically record temperatures over a period of time in a chart format. Alternatively, the placement of a US National Institute of Standards and Technology (NIST)-certified thermometer in the centre of the storage unit, adjacent to the vaccine, allows the unit's temperature to be read and documented twice a day. Records are then kept for a minimum of three years, as recommended in the US CDC guidelines [4]. If temperatures are found to be outside the recommended range, action must be taken immediately.

Storage equipment that features an automatic defrost system is highly recommended, as it prevents water, ice, frost or coolant leaks that could potentially harm vaccine samples. Refrigeration units provide efficient compression technology and forced-air circulation to maintain temperature uniformity throughout the cabinet. They also provide a tight and secure door closure through the use of spring-loaded, self-closing doors and effective door seals, to offer additional vaccine protection. Some units also supply 'Door Ajar' alarms that notify users when the cabinet door is not closed properly. Blown-in insulation that conforms to the unit's shape prevents cold air escaping, as well as internal temperature fluctuations due to exposure to ambient conditions. Many modern laboratory refrigerators and freezers also supply audio and visual alarms that alert users to temperature deviations, providing a real-time warning that cannot be ignored when, for example, preventative maintenance is required. Every clinic should be prepared with a recovery plan, in case of problems, that includes a refrigerator with a back-up generator in the event of, for example, a power cut or natural disaster. Today's refrigeration equipment can provide a full alarm function in case of power failure and a low battery alarm that displays when the alarm system battery backup is low.

▶ Best practice vaccine protection

The storage units selected should be of a high enough quality to negate the need for frequent maintenance and repairs, which can compromise vaccine quality due to cabinet 'downtime' and the transfer of samples to another location. Temperature should be recorded twice daily and any deviations from the optimum range should be reported. Studies have demonstrated that educating at least one staff member about correct monitoring and reporting of the refrigerator temperature significantly improves the maintenance of storage conditions, with fewer deviations from optimal temperature ranges going unreported [7, 8]. It is recommended that clinics should use dedicated refrigerators and freezers to store vaccines. Although combined refrigerator/freezer units are acceptable for vaccine storage if each compartment has a separate door, inconsistencies in temperature uniformity can arise, making combined units less suitable for the storage of any temperature-critical samples [9]. Clinics need to make allowances for the largest possible batch of vaccines that they will need to store at one time. This can be problematic as pandemics, e.g., the recent H1N1 (swine flu) outbreak, are not easily predicted. However, since individual doses need to remain 5 - 8 cm away from all walls, doors, drawers and cold air vents (as these are most likely to have temperature extremes), the footprint of the freezer needs to be chosen with care [8].

→ Conclusion

With organisations investing much time, effort and money to screen, develop and produce potentially life-saving vaccines, it is detrimental in terms of time and cost-efficiency if these vaccines lose effectiveness through incorrect storage. Maintaining constant conditions for vaccine storage is key to improving viability and protecting a population from health risks. Selecting the most efficient clinical cold storage equipment is therefore crucial for ensuring sample protection. The latest available refrigeration equipment enables much easier and more reliable monitoring and maintenance of ideal temperature conditions for vaccine storage, reducing the occurrence of temperature fluctuations to a minimal level. These in-built technological advances in storage units, in combination with a degree of education on quality control measures, can help to ensure that vaccines remain viable after storage, eliminating the need for costly losses and the implementation of revaccination programmes.

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The focus of this application note is on introducing different luciferase reporter assay types and the requirements they place on the luminometer. It also shows how luciferase reporter gene assays provide excellent performance using low assay volumes with normal 384-well and shallow well 384 plates.

Versatile Luciferases:
New Tools for Reporter Assays

Luciferase genes are commonly used as reporter genes in gene regulation studies. Reporter gene assays are used both in basic research for studying transcriptional regulation and cell signalling, and in drug discovery for screening and validating drug targets. The light output from luciferase reactions can be detected with luminometers that are suitable for reporter gene assays.

Thermo Fisher Scientific has developed a family of luciferase activity assays that use novel luciferase genes from *Cypridina* and *Gaussia* combined with the Red Firefly luciferase gene and the green shifted *Renilla* gene. The assay family provides both flash- and glow-type assays, which differ in the stability of luminescence signals and in sensitivity. Some of the assays utilise luciferases that are secreted into the culture media. This feature allows live monitoring of the reporter activity during cell growth, without a need for cell lysis. In addition, the assay family includes unique dual luciferase assays for multiplexing purposes. The dual assays include two luciferases: one for measuring the experimental luciferase activity and the other for measuring control activity for normalisation.

Assay Types

1. Glow-type luciferase assays

Glow-type luciferase reporter gene assays produce a very stable luminescence light signal that lasts for approximately one hour. As the light emission decays slowly, measurement does not have to be performed immediately after the addition of substrate. The stability of the light makes it possible to pipette all the assay reagents manually without need of reagent dispensers installed in the instrument. Glow-type assays are generally less sensitive than flash-type assays.

2. Flash-type luciferase assays

Flash-type luciferase reporter gene assays are in general more sensitive than glow-type assays. Luminescence signals are transient in flash-type assays, so the signal peak is reached soon after assay reagent addition. Therefore, flash-type assays often require the assay reagent to be added with automatic dispensers installed in the luminometer. Reagent dispensers in the instrument facilitate signal monitoring right from the start of the luminescence reaction and enable precise timing between dispensing and measurement from sample to sample. Pipetting the assay reagent manually increases sample-to-sample signal level variations especially in flash-type assays and miss the capture of true flash signal due to delay in measuring the signal after manual reagent addition.

3. Dual luciferase assays

Thermo Scientific Dual luciferase assays are highly sensitive flash-type assays for multiplexing. The one-step assays are based on having two luciferases emitting luminescent light at spectrally distinct wavelengths. The signals can easily be separated using a luminometer equipped with high transmission filters optimised for the assays. This kind of dual assay eliminates the need for signal quenching between the measurement of two luciferases. Thus, both luciferase signals can be detected simultaneously from a single sample with a single reagent addition. As these dual assays are flash-type assays, the reagent dispensers installed in the luminometer act to ensure optimal performance and sensitivity.

Luminometers

Luminescence light output from luciferase reporter gene assays can be quantified using, for example, Thermo Scientific Varioskan Flash or Luminoskan Ascent microplate luminometers (Figure 1). These luminometers have excellent sensitivity in luminometry, both in glow- and flash-type assays. In addition, the Varioskan Flash can measure luminescence spectra as well as other detection technologies.

Reagent dispensers & Optical filters

Dispensers are essential in flash-type assays, which require measurement soon after the addition of substrate, and precise timing between dispensing and measurement from sample to sample and from day to day. Dispensers installed in the luminometer make the reaction triggering easy, fast and accurate, not only for flash-type assays, but also for glow-type assays. Both the Luminoskan Ascent and Varioskan Flash can be equipped with up to three reagent dispensers. In addition, both instruments can be equipped with optical filters for measuring dual luciferase assays. The filters have been specifically optimised to separate the two luciferase signals as efficiently as possible for the Pierce Dual Luciferase Assays and, therefore, they ensure excellent performance.

Materials and Methods

The study of this application note focuses on performing flash-type Luciferase Reporter Assays in low assay volumes with normal 384 and shallow well 384 plates.

- Instrument: Varioskan Flash multimode microplate reader equipped with a luminometric measurement module and an onboard dispenser for automatic reagent addition
- Kits: Flash-type Luciferase Reporter Assay Kits for *Gaussia*, *Cypridina*, *Renilla* and Firefly luciferases
- Microplates: white 384-well OptiPlate (Perkin Elmer, Cat. No. 6007299) and white shallow well 384-well plate (Thermo Scientific NUNC, Cat. No. 264706)

Luciferase samples were diluted in the Cell Lysis buffer provided in the kit to create a concentration series of over eight orders of magnitude. Aliquots of 4 µl (normal 384-well plate) or 1 µl (shallow well 384 plate) of each dilution were added into the microplate wells. Instrument control software was programmed to add 20 µl or 5 µl of luciferase assay reagent with a dispenser and to measure the signal kinetically for 15 seconds at a sampling rate of ten readings per second. Each well was dispensed and measured before proceeding to the next well. Luminescence signals were integrated from kinetic curves for different time periods. Additionally, individual signals and signal maxima were collected for comparison of different time points. Assay sensitivity and dynamic range were calculated for each data set.

Figure 1. Optimal microplate readers for Pierce Luciferase Reporter Assays: Varioskan Flash (on the right) and Luminoskan Ascent (on the left).

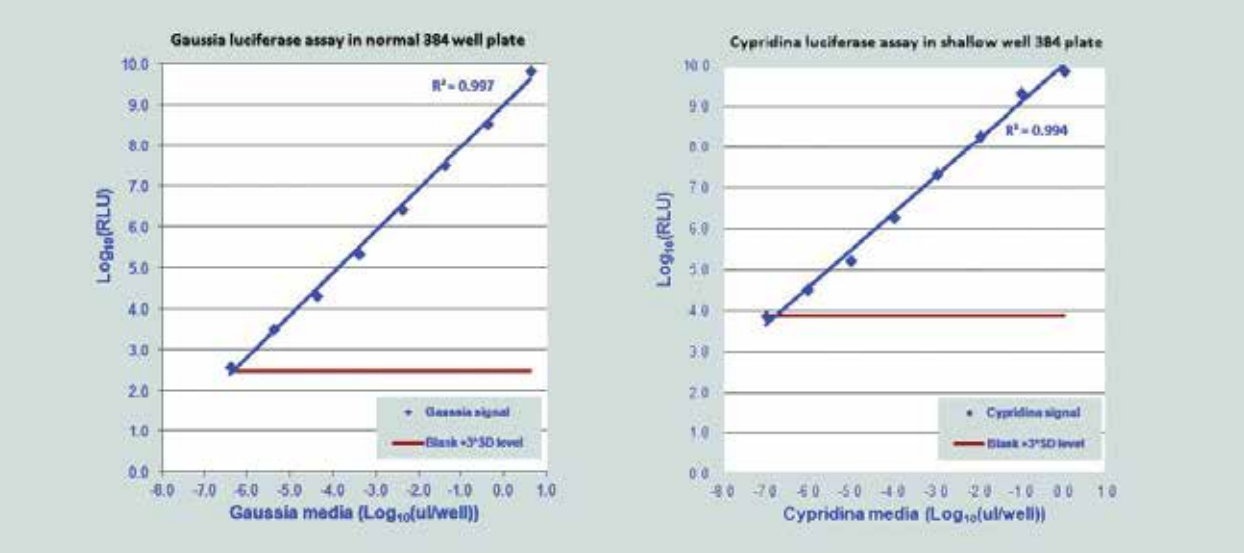

Figure 2. Typical standard curves of *Gaussia* and *Cypridina* luciferase flash assays with different plate formats

Results

The Pierce Luciferase Reporter Assays and the Varioskan Flash provide very high performance and enable use of very low assay volumes with normal 384-well and shallow well 384-well plates. All luciferase reporter assays show perfect linear response over a wide concentration range with both normal and shallow well plates. Real assay dynamics reaches eight orders of magnitude with the *Gaussia* and *Cypridina* flash assays (Figure 2). The *Renilla* flash assay also shows a remarkably large dynamic measurement range. Flash assay with Red Firefly luciferase shows a somewhat reduced dynamic range, about 5 to 6 orders of magnitude.The Varioskan Flash can automatically adjust the photomultiplier gain to increase the assay dynamic range to eight orders of magnitude. This unique feature means that the luciferase samples can have any luciferase concentration over a large 100 million fold concentration range. Thus the samples can always be measured without additional dilution steps.

Figure 3 illustrates the flash-type reaction kinetics of *Gaussia*, *Cypridina*, *Renilla* and Firefly luciferases. All the assays reach the light emission maximum in a few seconds after the reaction has been started. *Cypridina* and *Renilla* assays show a fairly stable light emission for several seconds, and the *Gaussia* assay shows about 3% signal decay per second after reaching maximum. The firefly flash assay represents the fastest flash reaction where the signal decays about 20% per second during the first couple of seconds.

Using automatic dispensers in the luminometer for reagent addition is highly recommended to ensure exact timing in the measurements, especially in *Gaussia* and Firefly assays. If the luminescence reaction is initiated by manual pipetting, it may lead samples to be measured in different time points of the kinetic reaction, thus increasing sample-to-sample variations.

Assay sensitivity is dependent on the measurement integration time, but its effect is not very strong. About a one second measurement time provides good sensitivity, but a few seconds more may be required for the best possible sensitivity. The *Cypridina* assay produces the most stable light emission and, therefore, it is recommended to integrate the signal somewhat longer than with assays with a more unstable signal. The Firefly flash reaction shows the most unstable kinetics and therefore the recommended integration time is the shortest. A usable integration time range is a range where changing the integration time has only a minor effect on the assay performance. When using shallow well 384 plates with smaller

Recommended Integration Times

	Normal 384-well plate		Shallow well 384-well plate	
Luciferase	Optimal time (s)	Usable range (s)	Optimal time (s)	rUsable ange (s)
gaussia	1.5	1-1.5	7	4-7
cypridina	5	1-5	7	3-7
green renilla	2	1-2	10	4-10
red Firefly	1	0.5-1	1.3	0.5-1.3

assay volumes, it is recommended to use longer integration times than with normal 384-well plates with higher assay volumes (See Table 1).
Even though this application note only presents the results of assays performed in a 384-well plate format, measurements in a 96-well plate format provide excellent performance as well. The performance of the Luminoskan Ascent with these luciferase reporter assays is also comparable to the Varioskan Flash results presented here.

Conclusions

- Pierce Luciferase assays provide an extremely wide dynamic range for measuring both low and high luciferase expression levels without concentrating or diluting samples.
- The Varioskan Flash detects luciferase activities over a dynamic range of about eight orders of magnitude with the automatic gain adjustment feature of the instrument.
- Using 384-well plates (either normal or shallow well) enables reducing both sample and reagent consumption without sacrificing assay performance. Use of shallow well 384 plates enables performing the assays in a total volume of about 6 µl (1 µl sample + 5 µl assay reagent).
- Highly sensitive flash-type assays can easily be performed with a microplate luminometer equipped with an automatic dispenser.
- Both inter- and intra-assay variations can easily be minimised using automatic luminometer dispensers, which enable precise timing of the reaction start and signal collection.

Different luciferase reporter gene assays require different features and capabilities of the luminometer. The benefits of using Thermo Scientific luminometers for performing Pierce Luciferase Reporter Assays include the following:

- Excellent luminometric performance in glow- and flash-type assays
- Onboard dispensers for reagent addition:
  - follow-up of fast kinetics of flash assays
  - precise timing between dispensing and measurement in flash assays
  - convenience and accuracy for flash and glow assays
- Optimised filters for measurement of dual assays
- Flexible and easy protocol setup and data processing with PC software

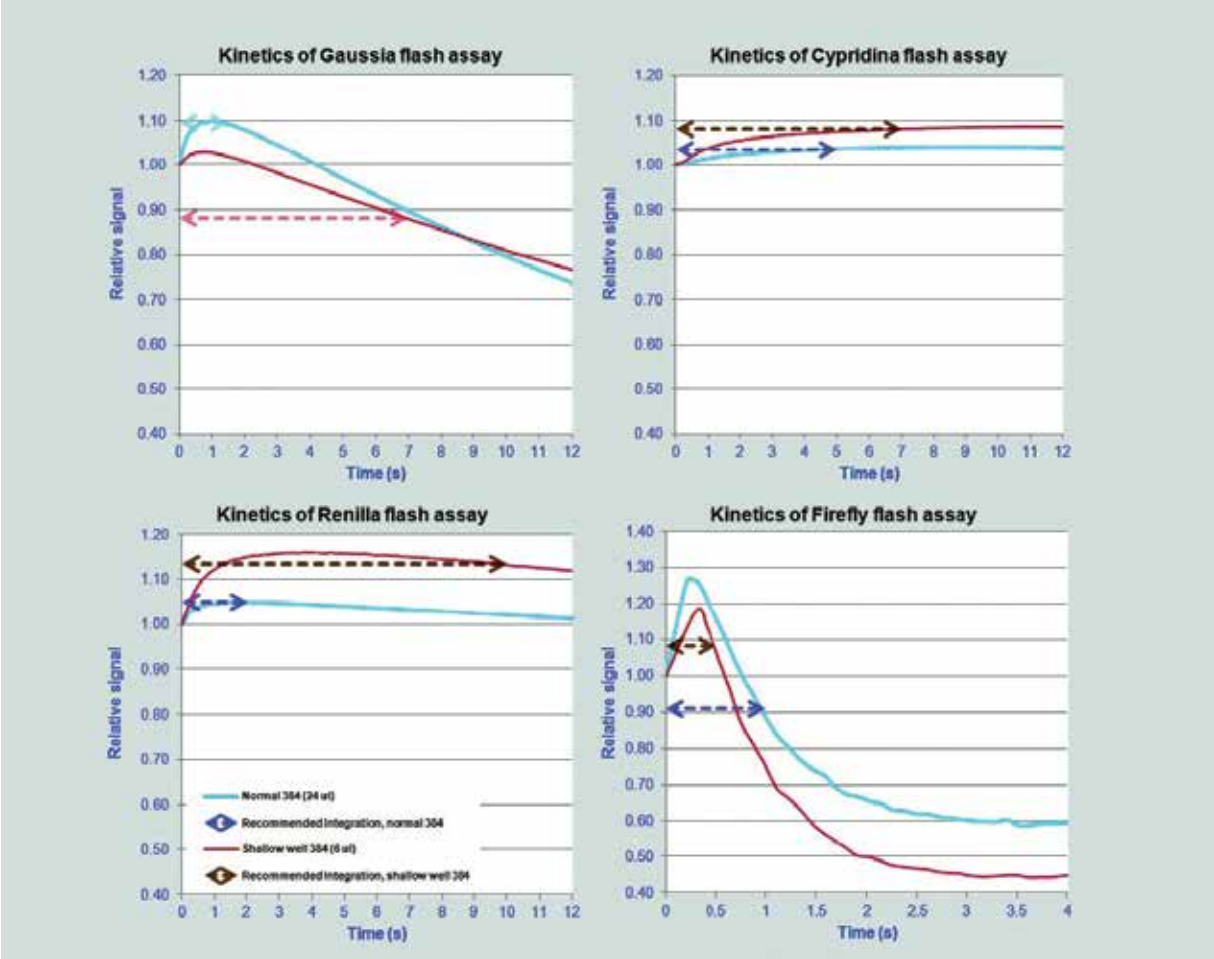
Kinetics of Gaussia flash assay
Kinetics of Cypridina flash assay
Kinetics of Renilla flash assay
Kinetics of Firefly flash assay
Relative signal
Time (s)
Normal 384 (24 ul)
Recommended integration, normal 384
Shallow well 384 (6 ul)
Recommended integration, shallow well 384

Figure 3. Luciferase reaction kinetics of flash-type assays measured both in a normal 384-well plate and a shallow well 384 plate. *Renilla* and Firefly luciferases were measured from a cell lysate. *Gaussia* and *Cypridina* were secreted to the growth medium and measured directly from the medium without a cell lysis.

tech note

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ClipTip interlock technology: *the next generation for pipettes*

The most common type of pipettes in the lab are air displacement pipettes. An airtight seal between the pipette and the tip is crucial for the pipette´s functionality, and any compromise on the sealing affects pipetting performance. Traditionally air displacement pipettes have relied on friction between the pipette and the tip to form a seal. The sealing is dependent on many factors including the tip attachment force used and might also be compromised through general use or when touching the vessel wall during pipetting. With the new Thermo Scientific F1-ClipTip Pipetting System the attachment of tips is accomplished with an interlock technology between pipette and tip.

Breakthrough ClipTip Technology

ClipTip interlock technology utilises flexible clips positioned evenly around the top of the tip. During attachment, the unique tip fitting shape opens the clips allowing it to pass over the fitting flange and return to closed position. The clips lock the tip behind the flange creating a complete seal with the sealing ring (figure 1). In addition, the lock ensures the tip will not loosen compromising the seal, or even potentially fall off during routine pipetting or touch-off.

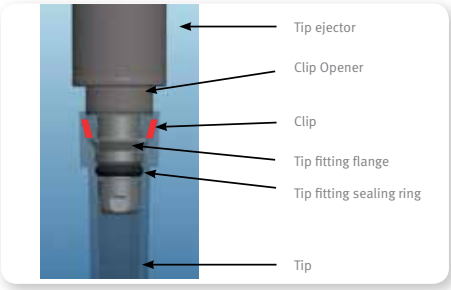

Figure 1: Clips closed behind the flange. The tip is sealed.

Test set-up

In this technical note we demonstrate the benefits of the revolutionary ClipTip interlocking tip attachment technology by pipetting into a 96-well plate as an example.

Test pipettes and tips:

- Thermo Scientific F1-ClipTip 12-channel pipette (30-300 µl) with ClipTip 300.
- 300µl 12-channel pipettes from Manufacturers A and B with manufacturers' tips recommended for the particular pipette as well as generic tips. The pipettes had friction based tip sealing mechanisms.

Test method:

- Tip attachment was achieved with constant forces using a machine to press the tested pipette downward against the tip rack box with a lever. The lever was equipped with a weight, which directed a constant downward force to the test pipette. This procedure simulated the tip attachment procedure with a known force. The used attachment weight forces were 3.8kg, 4.8kg and 6.3kg (37.3N, 47.1N and 61.8N respectively).
- After tip attachment 100 µl of green dye solution was dispensed into 96-well microplates using the reverse pipetting technique. After each dispensing the tips were touched against the wall of the wells to wipe off possible droplets on the outside of the tip. It was recorded if tips fell off. After filling two microplates, 100 µl was aspirated and the amount of liquid was measured gravimetrically. Precision values (CV%) were calculated for each attachment force used.

Results

1. Tip attachment

Attachment force 3.8 kg was sufficient to attach tips to all pipettes tested. However, tips from Manufacturer B and generic tips fell off during pipetting when 8 rows of the microplate had been dispensed. Figure 2 shows ClipTip tips (a) and Generic pipette tips (b) after filling two 96-well microplates. The ClipTip pipette tips did not fall off, and secured an equal liquid level in all 12 tips.

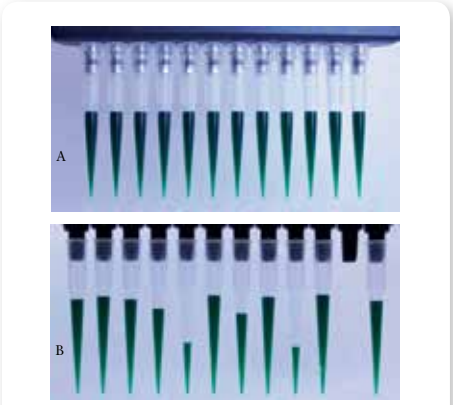

Figure 2. ClipTip tips (a) and Generic tips (b) after dispensing into two 96-well microplates. The tip attachment force used was 3.8 kg.

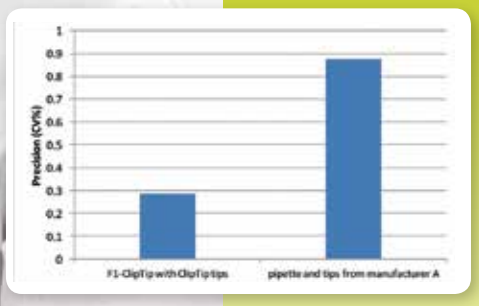

Figure 3. Dispensed volumes of F1-ClipTip 12-channel pipette and Manufacturer A's 12-channel pipette after dispensing into two 96-well microplates. The set volume was 100 µl and the tip attachment force used was 3.8 kg.

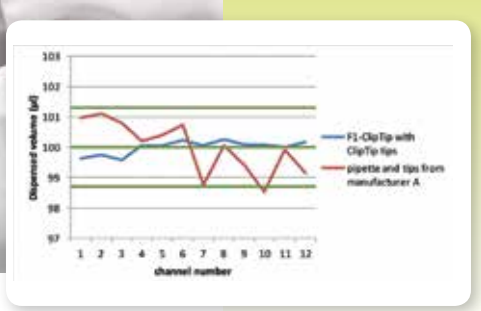

Figure 4. Precision values with 12-channel pipettes after dispensing into two 96-well microplates. The set volume was 100 µl and the tip attachment forces used were as 3.8 kg.

The Generic tips had one tip fall off, and obtained uneven liquid levels in the remaining tips. In order to keep tips attached the force needed to be increased from 3.8 kg to 4.8 kg. The tips of manufacturer A stayed attached, but the variation between dispensed volumes of different channels was higher than with F1-ClipTip (Fig. 3). This variation could not be observed visually.

2. Precision

Precision value of manufacturer A was 68% higher than with F1-ClipTip system (Figure 4) after dispensing into two 96-well microplates. The figure shows that with F1-ClipTip the variation between channels was lower than with a pipette with a friction based tip fitting mechanism. With friction based mechanisms the precision varied with the tip attachment force used.

Summary

An optimal pipette and tip system increases confidence in reproducibility, reduces forces required to attach and eject tips, and secures the best possible accuracy and precision. In microplate applications, demand for optimum pipetting performance, and comfort is even greater due to higher number of repetitions and samples. Compared to Manufacturer A and B, the F1-ClipTip pipetting system demonstrates excellent precision and minimal variation between dispensed volumes of different channels. Results show that pipetting performance of the F1-ClipTip system is not affected by the tip attachment force used. All tips remained attached and variation between channels was minimal. In addition, tip attachment did not require banging or rocking.

With friction based tip attachment systems the seal between the tip and the tip cone is accomplished by friction between the pipette and tip. In this experiment impaired precision after dispensing and touching tips to the walls of a microplate was observed. This is likely due to loosening of the tips, which affects the seal. The sealing may be tight enough to avoid visible leakage, but not tight enough to give optimal pipetting results. In the worst case the tips fell off in the middle of pipetting. In a real research application this would mean repeating the experiment.

Loss of samples and/or reagent, as well as time wasted, have a large impact on daily research and can especially be seen with microplate applications. Dropped tips may also present a severe contamination risk particularly in clinical applications. With Manufacturer A and B more effort was needed to attach the tips firmly, and prevent tips getting loose or dropping off.

The complete seal between the sealing ring and the ClipTip tips guaranteed optimal pipetting performance as demonstrated by the excellent precision. The unique interlocking tip attachment mechanism ensured that the tips stayed firmly attached through the entire application. These innovative features make F1-ClipTip Pipetting System an excellent choice for microplate applications by saving valuable samples/reagents as well as time and resources while increasing confidence in pipetting results. In addition, the ergonomic benefits of this system reduce the risk of Repetitive Strain Injuries while pipetting.

Water-cooled condenser option

for Thermo Scientific Ultra-Low Temperature Freezers

Freezer farms with ultra-low freezers are very common in academic institutions, sample storage areas, biopharma sites and many other locations where ultra-low temperatures are required to protect samples. Heat discharge is a normal function of compressor driven units. The heat generated can cause significant strain on HVAC systems. HVAC is taxed to the limit, which causes additional cost to run the entire building system.

What is the best time or application to consider putting water cooled condenser into a building?

- New construction projects. (Design Phase)
- Renovation
- Locations that have existing recirculating water systems or chilled water source
- Multiple freezers or freezer farms

Advantages to water cooling condensers

- Overall energy savings potential
- 50% heat reduction to the laboratory or surroundings*
- Reduced impact on the HVAC system
- More comfortable work environment in the lab yields more productive work
- Heat reduction in the laboratory reduces potential risk to heat sensitive products or chemicals

How do chilled condensers function?

The air-cooled condenser looks like a small radiator (like in your car). The hot gas from the high stage compressor is circulated through the air-cooled condenser where a fan blows across it to remove the heat and blow it into the room in air cooled condenser. A water-cooled condenser goes in place of the air-cooled condenser and instead of a fan blowing across it, coils of water is circulated around it, which absorbs most of the heat and circulates water back to the water tower or chilled water reservoir system. A graphic example of a water-cooled condenser is shown in figure 1.Ultra-low freezers using chilled water condensers must have water flowing through the condenser at all times. Chilled water systems likely will require periodic maintenance. The freezers can be protected by the connection to city or house water during the maintenance process.The following procedure is recommended to switch between water used for cooling. Connections must comply with local building codes. The conversion process assumes chillers and house water are available. Through water cooling, the heat rejection rate of a ULT can be reduced by 70 to 80%[1].

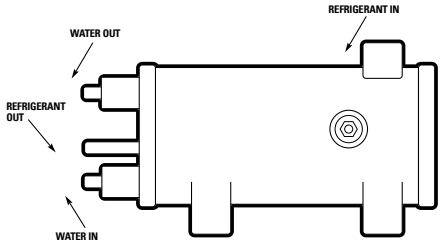

Figure 1. Graphical example of a water-cooled condenser.

Water cooling condensers offer an alternative to the extra heat load on the HVAC system. Water cooling **reduces heat** to the surroundings by approximately **50%.**

Process to switch from chilled water source to house water source

- Check water pressure in both systems. Pressure value should be x value.
- Purge the house water line to remove air or particulates in the house water lines.
- Check the water pressure: It must be at target pressure for house systems to protect the ultra-low temperature freezers.
- Switch the valve to house water.
- Turn off chilled water source.
- Monitor the temperature and water pressure on the house line.

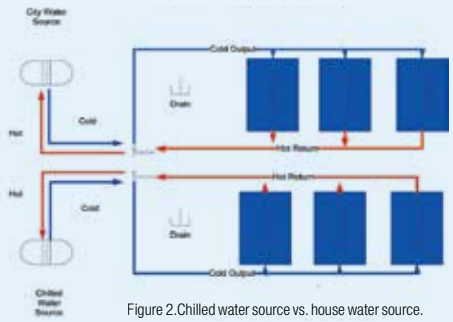

Figure 2.Chilled water source vs. house water source.

Process to switch from house water source to chilled water source

- Check water pressure in both systems. Pressure value should be x value.
- Check the water flow rate. If there is no information on water flow rate, check the first stage discharge pressure.
- Note the water pressure, inlet water temperature and first stage discharge pressure
- Purge the chilled water line to remove air or particulates in the chilled water lines.
- Check water pressure. Must be at target pressure for chilled systems to protect the ultra-low temperature freezers.
- Switch the valve to chilled water .
- Turn off house water source.
- Monitor the temperature and water pressure on the chilled water line.

Novel glycan column technology

for the LC-MS analysis of labelled & Native N-Glycans Released from Proteins & Antibodies

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Methods

Sample Preparation

Release native glycans from glycoproteins with PNGase F enzyme. Conjugate the released glycans with 2-amino benzamide (2AB) label group using the reported procedure of Bigge *et al*.[4] Here, 2-AB A1 (P/N GKSB 311), 2-AB A2 (P/N GKSB 312), and 2-AB A3 (P/N GKSB 314) were purchased from Prozyme® (Hayward, CA). Prior to analysis, dissolve samples in 100% buffer (100 mM ammonium formate, pH = 4.4) and dilute further with acetonitrile to make 30% buffer and 70% acetonitrile.

Liquid Chromatography

All the glycans were separated using a Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS system and either a Thermo Scientific™ Dionex™ UltiMate™ FLD-3400RS Rapid Separation Fluorescence Detector or MS detector.

Mass Spectrometry

MS analysis was performed with a Thermo Scientific™ Q Exactive™ Benchtop LC-MS/MS in negative ion mode at the following settings: MS scan range 380–2000. FT-MS was acquired at 70,000 resolution at m/z 200 with AGC target of $1e^6$; and DDA MS2 acquired at 17,500 resolution at m/z 200 with AGC target of $2e^5$.

Data Analysis

SimGlycan® software (PREMIER Biosoft) was used for glycan identification and structural elucidation data analysis.[5] SimGlycan software accepts raw data files from Thermo Scientific™ mass spectrometers and elucidates the associated glycan structure by database searching and scoring techniques.

Results

Separation of Labelled Glycans Based on Charge, Size, and Polarity

The GlycanPac AXH-1 column can be used for qualitative, quantitative, and structural analysis as well as characterisation of uncharged (neutral) and charged glycans present in proteins. Figure 1 shows bovine fetuin on the GlycanPac AXH-1 (1.9 µm, 2.1 × 150 mm) column using fluorescence detection. The separation and elution of glycans are based on charge: the neutral glycans elute first, followed by the separation of acidic 2AB labelled N-glycans from monosialylated, disialylated, trisialylated, tetrasialylated and finally pentasialylated species. Glycans of each charge state are further separated based on their size and polarity. The retention time of each glycan charge state was confirmed using 2AB labelled glycan standards (as shown in Figure 2). Separation of glycans is based on charge, size, and polarity, which provides significant structural and quantitative information. The chromatographic profiles shown in Figures 1 and 2, detected by fluorescence detection, provide qualitative information about the separation of N-glycans. The structure of glycans present in each peak was determined from the LC-MS study using the GlycanPac AXH-1 (1.9 µm) column as shown in the following section.

LC-MS and LC-MS/MS Analysis of 2AB Labelled N-Glycan Using GlycanPac AXH-1 Column

The coupling of the GlycanPac AXH-1 column to MS was also explored. This is particularly attractive as MS, with it's ability to provide structural information, enables in-depth analysis of complex glycans. 2AB labelled N-glycans from bovine fetuin were separated on the GlycanPac AXH-1 column and analysed on a Q Exactive mass spectrometer. Data-dependant MS/MS spectra were acquired on all precursor ions (z< 2) and

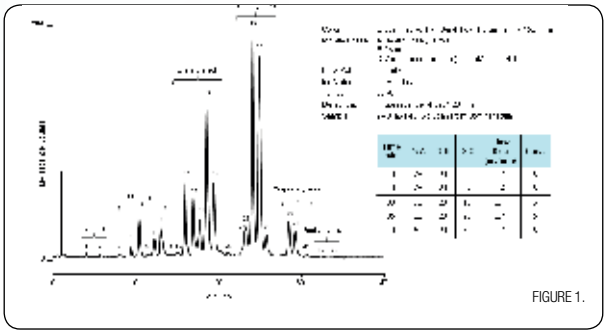

FIGURE 1.

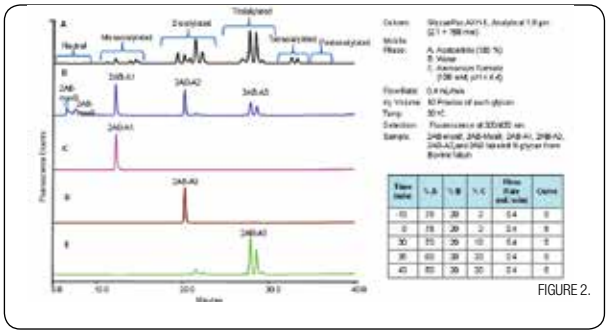

FIGURE 2.

SimGlycan software was used for glycan structural elucidation. A representative example of the analysis is shown in Figure 3. The detailed structural information obtained from the MS/MS data further validated the ability of the GlycanPac AXH-1 column to separate glycans based on charge, size, and polarity. However, coelution of different charge state glycans (Figure 4) is common with other commercially available HILIC columns.

LC-MS Analysis of Native Glycans Released from Proteins

The GlycanPac AXH-1 column is well suited for high-performance LC/MS separation and analysis of native glycans from MAbs and other proteins. Analysing unlabelled glycans not only eliminates the extra reaction step and cumbersome cleanup methods during labeling, but also retains the original glycan profile without adding further ambiguity imposed by the labeling reaction. Figure 5 shows the LC/MS analysis of native N-glycans from Bovine fetuin using the GlycaPac AXH-1 column (1.9 µm). The native glycans were separated based on charge, size, and polarity. Using an ammonium formate/acetonitrile gradient highly compatible with MS detection, the separation enables excellent MS and MS/MS fragmentation data for accurate confirmation of the glycan structure of each chromatographic peak. Native glycan profiles are significantly different from the profile of fluorescently labelled glycans, especially higher sialic acid glycans. However, fluorescently labelled glycans generally provide better and more MS/MS fragmentation peaks. The GlycanPac AXH-1 column is useful for the analysis of both native and labelled N-glycans, depending on the amount of sample available. If the amount of the sample is not extremely limited, analysis of unlabelled glycans using the GlycanPac AXH-1 is highly feasible.

Structural Analysis of N-Glycans from Antibodies by LC-MS Using GlycanPac AXH-1 Column

Antibody research has gained significant interest as a part of the development of protein biotherapeutics. Glycosylation of antibodies is a prime source of product heterogeneity with respect to both structure and function. Variation in

FIGURE 1: Separation of 2AB labelled N-glycans from Bovine fetuin by charge, size and polarity.

FIGURE 2: Comparison of 2AB labelled N-glycans standards and 2AB-N-glycans from fetuin.

FIGURE 3: LC-MS analysis of 2AB labelled N-glycans from Bovine fetuin by the GlycanPac AXH-1 (1.9 µm) column with MS detection.

FIGURE 4: LC-MS analysis of 2AB labelled N-glycans from Bovine fetuin by a commercial amide HILIC column (1.7 µm) with MS detection.

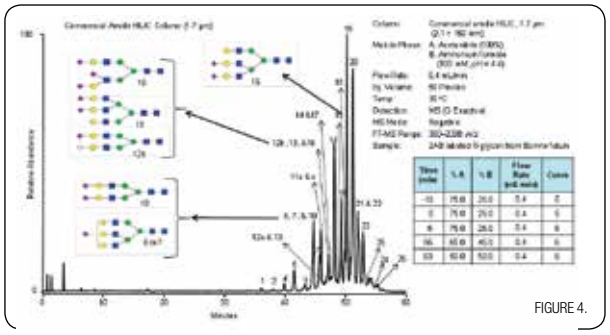

FIGURE 4.

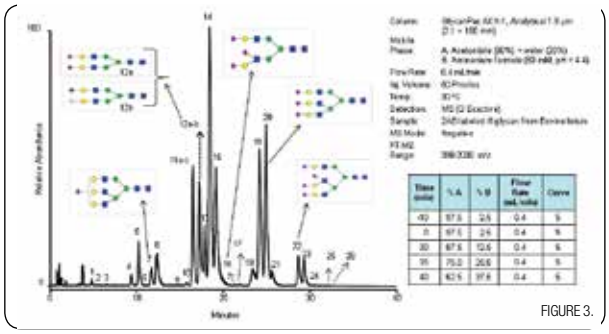

FIGURE 3.

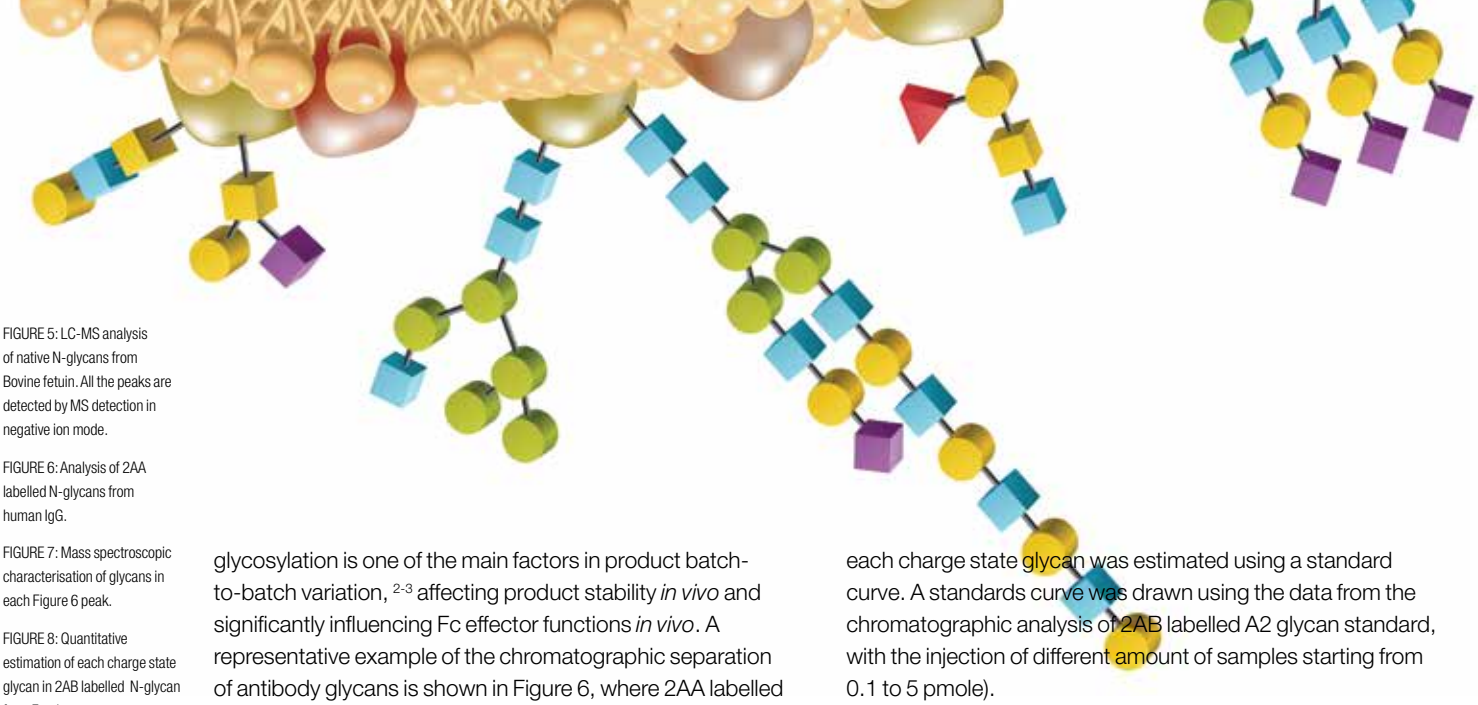

FIGURE 5: LC-MS analysis of native N-glycans from Bovine fetuin. All the peaks are detected by MS detection in negative ion mode.

FIGURE 6: Analysis of 2AA labelled N-glycans from human IgG.

FIGURE 7: Mass spectroscopic characterisation of glycans in each Figure 6 peak.

FIGURE 8: Quantitative estimation of each charge state glycan in 2AB labelled N-glycan from Fetuin

glycosylation is one of the main factors in product batch-to-batch variation, [2-3] affecting product stability *in vivo* and significantly influencing Fc effector functions *in vivo*. A representative example of the chromatographic separation of antibody glycans is shown in Figure 6, where 2AA labelled N-glycans from IgG were separated using the GlycanPac AXH-1 column (1.9 µm). Characterisation of glycans in each peak was performed by LC-MS/MS and results are shown in Figure 7. Three different glycan charge states were found in this human IgG; the majority of glycans are neutral or monosialylated, with minor amounts of disialylated glycans. Separation of glycans based on charge provides advantages compared to other commercially available HILIC columns.

Quantitative Determination of Glycans Based on Charge

Quantitative analysis of each glycan is essential for quick assessment of glycan variation in protein batch comparisons and for comparison of diseased to normal cell glycosylation profiles. In addition, quantitative analysis of glycans separated based on charge state also provide a tool for calculating the relative amounts of different sialic acid linkages after enzymatic digestion with silidase S and sialidase A. Figure 8 shows the quantitative analysis of 2AB labelled N-glycans based on charge the using GlycanPac AXH-1 column (1.9 µm) with fluorescence detection. The relative amount of each charge state glycan was estimated using a standard curve. A standards curve was drawn using the data from the chromatographic analysis of 2AB labelled A2 glycan standard, with the injection of different amount of samples starting from 0.1 to 5 pmole).

Conclusion

- The GlycanPac AXH-1 column separates glycans with unique selectivity based on charge, size, and polarity not possible with commercial HILIC columns.
- LC-ESI-FTMS or FT-MS/MS analysis of both native and labelled glycans from proteins and antibodies were carried out successfully using GlycanPac AXH-1 columns.
- The GlycanPac AXH-1 column is useful for both high-resolution charge-based separation and easy quantification of glycans.
- The GlycanPac AXH-1 columns are compatible with various MS instruments.
- These new columns have high-chromatographic efficiency and excellent column stability.
- The GlycanPac AXH-1 column is also useful for the separation of reduced O-glycans from proteins and mucins.
- The GlycanPac AXH-1 column is useful for the analysis of charged and neutral glycosylaminoglycans and glycolipids.

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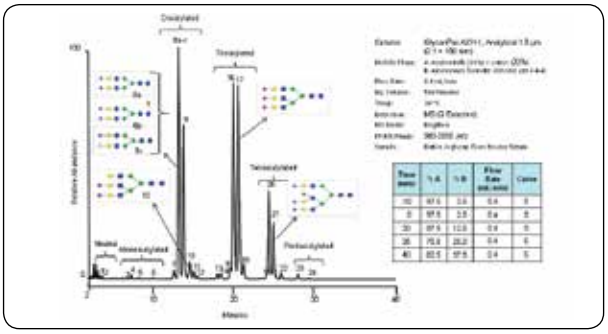
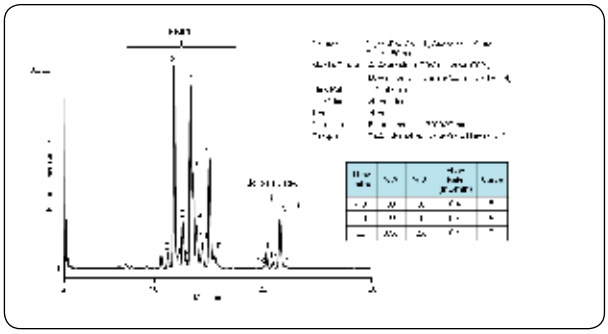
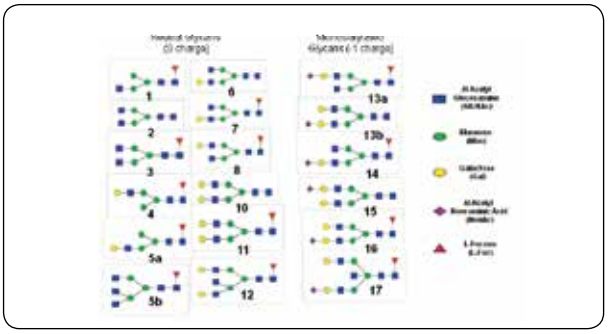
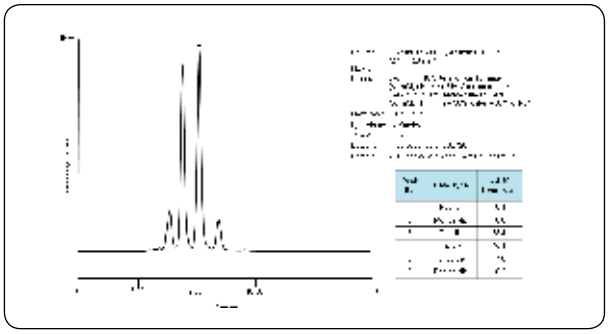
Acknowledgements

We would like to thank Mark Tracy, Yoginder Singh, Jessica Wang, and Patrick K. Bennett from Thermo Fisher Scientific for their help and permission to use their UHPLC and MS instruments.







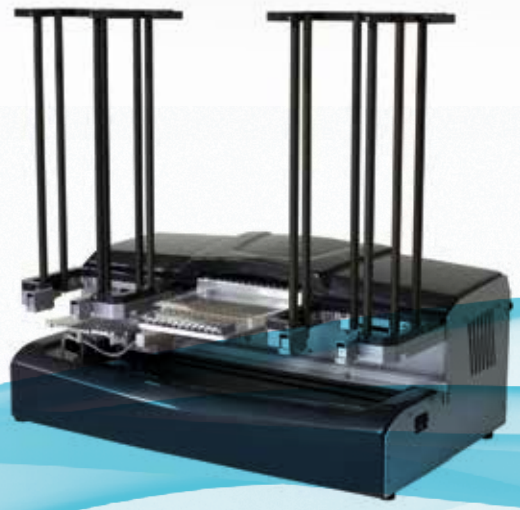


flexible low volume liquid handling

Evaluation of the Formulatrix Tempest at EMD Serono: A flexible low volume liquid handler with two axis gradient dispensing capability

Vikram Shankar*, Adam Shutes, Bill Griffin, Brian Healey Lead Discovery Technologies, EMD Serono Research Institute, Rockland, MA, 02370. *vikram.shankar@emdserono.com

Liquid handling instrumentation which provides precise and flexible dispensing over a range of volumes and different components is core to a fully functional assay development, hit discovery and characterisation lab. At EMD Serono, we use liquid dispensing (50 µl to 200 nl) in a limited number of tasks, such as regular assay component dispensing (around 15-70 µl), as well as low volume dispensing (200 nl). Other aspects of assay development & biochemical characterisation of inhibitors are performed in a manual and labour intensive manner.



Introducing the Formulatrix Tempest in our workflow has allowed us to improve the current technique not only in terms of efficiency but also accuracy and precision. Unique to the Tempest is its new microfluidic technology that relies on independent channel control over all the 96 nozzles, thereby allowing one to dispense any volume from any input into any well. We evaluated this diversity for a wide range of assays from those requiring factorial dispensing, like assay design and modality of inhibition studies, to applications like assay miniaturisation that use fixed volume dispensing.

Tempest

The Tempest is a flexible and compact liquid dispenser that uses state of the art microfluidics to dispense any volume from any input into any well.

It uses positive displacement to dispense liquids with minimal waste. It has a dispensing range of 200nl to no upper limit and a very low non-recoverable dead volume (~40µl). Typical dead volumes are ~400µl and can be reduced to ~100µl using tip dispensing. It is accurate, precise, fast and is compatible with 96, 384, 1536 well micro plates.

Tempest accuracy

We analysed the accuracy of the Tempest in two formats
A fixed volume miniaturised assay comprising of 2µl enzyme & 3µl substrate.A variable volume assay that requires different volumes of 8 different enzymes.With both formats, we found the CV's for the whole plate to be within the acceptable range of 5%.

5ul Assay		
Avg	Std dev	CV
0.390	0.003	0.818
0.388	0.003	0.896
0.390	0.003	0.739
0.389	0.003	0.805
0.392	0.004	0.993
0.390	0.004	1.018
0.390	0.004	0.950
0.387	0.004	0.974
0.390	0.003	0.754
0.390	0.003	0.714
0.389	0.003	0.801
0.389	0.003	0.789
0.387	0.003	0.761
0.386	0.003	0.755
0.388	0.004	1.011
0.386	0.003	0.866

Variable Volume Assay (CV's)	
Kinase I	1.318
Kinase P	1.122
Kinase A1	1.133
Kinase F	1.296
Kinase I	1.508
Kinase P	1.550
Kinase A1	2.519
Kinase F	2.374
Kinase FL	1.885
Kinase R	3.109
Kinase S	2.109
Kinase V	1.905
Kinase FL	1.820
Kinase R	1.584
Kinase S	1.721
Kinase V	2.085

Workflow comparison

Our current workflow for establishing assay parameters involves 3 separate steps.

a) Titration to determine the enzyme concentration for a 30% conversion. (Fig 1)

b) Kinetic run to determine the ATP Km of the enzyme. (Fig 2)

c) Final titration to confirm the established parameters. (Fig 3)

These resource intensive steps can be eliminated with the Tempest, which by virtue of its independent channel control allows a factorial dispense of ATP and enzyme. The resulting matrix design enables us to attain the assay parameters in a single step (Fig 4,5).

Modality of inhibition

We evaluated the Tempest's efficiency in modality of inhibition experiments. By dispensing a gradient of ATP down the plate we studied the response of two in-house competitive inhibitors.

Data

Figures 6 & 7 show IC50 curves for inhibitors (A & B) at distinct ATP concentrations. IC50 values at other concentrations are given in table 1. It can be seen from Fig 8 and 9 that for both Inhibitor A and Inhibitor B, the potency of inhibition decreases with increase in ATP concentration, a characteristic of Competitive Inhibition.

Figure 1 : Kinase A enzyme titration at 100uM ATP

Figure 2: Kinetics for Kinase A

Figure 3: Kinase A enzyme titration at 15uM ATP

Figure 4 : Kinetics for Kinase A (TEMPEST)

Figure 5 : Kinase A enzyme titration at 15uM ATP (TEMPEST)

Figure 6 : Inhibitor A at 15 and 250 uM ATP

Figure 7 : Inhibitor B at 15 and 250 µM ATP

Figure 8 : Competitive inhibition of Kinase A by Inhibitor A

Figure 9: Competitive inhibition of Kinase A by Inhibitor B

Figure 10: A375 cells dispensed manually

Figure 11: A375 cells dispensed using Tempest

Table 1: IC50 values of both inhibitors at various ATP concentrations

Figure 1.

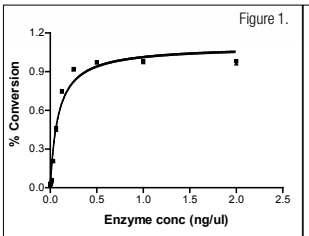


Figure 2.

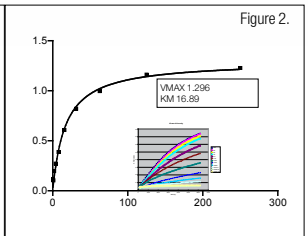


Figure 3.

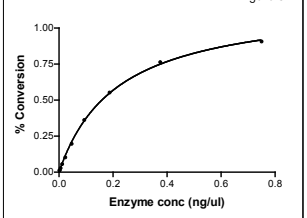


Figure 4.

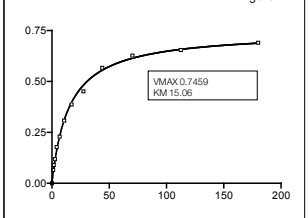


Figure 5.

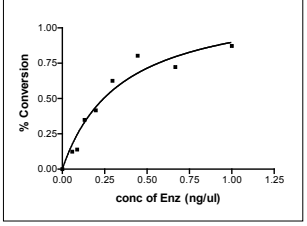


Figure 6.

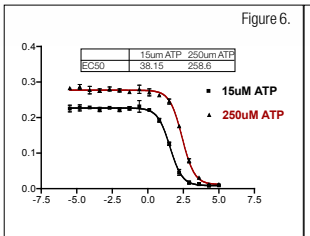


Figure 7.

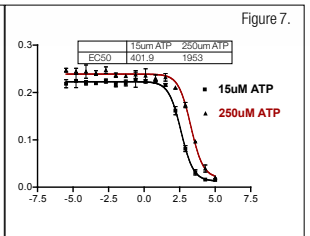


Figure 8.

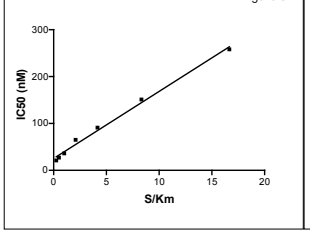
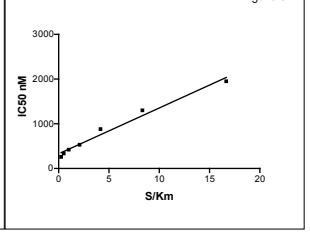


Figure 9.



Inhib A IC50 (nM)	ATP (uM)	Inhib B IC50 (nM)
258	250	1953
151	125	1300
91	62.5	882
65	31.25	530
36	15	402
27	7.5	337
21	3.75	258
10	1.75	119

Table 1.

Figure 10.

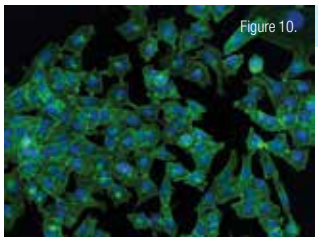
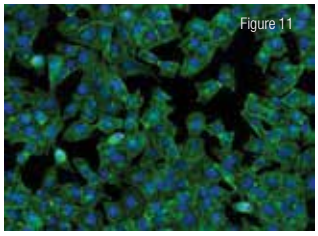


Figure 11



Cell dispensing

We further evaluated whether operation of the diaphragm during dispensing had an effect on cells. As shown in Fig 10 & 11, we saw no morphological changes in cells dispensed with the Tempest when compared to manual dispense.

Summary

Low volume pipetting technology is key to our workflow and is of utmost importance. Here we have highlighted the improved efficiency the Tempest provides to our workflow. Other key features include an easy USB connectivity, a user friendly software and automation capability. ELISA, PCR, HTS, DNA/RNA research are some of the other applications that the Tempest can support.

product focus

Thermo Scientific™ Barnstead™ Type 1 water purification systems

Why is UV intensity monitoring important for ultrapure water?

UV intensity monitoring is an innovative technology designed to ensure that the total organic carbon (TOC) reading is accurate, providing superb reliability for ultrapure water. The monitoring and display of the TOC content in product water has become increasingly important as biochemical methods become more sensitive to low levels of organics. In addition to visualising the resistivity of the ultrapure water, you need to quantify the amount of organic impurities in the water. Organic-free water is critical to applications that are sensitive to organics, such as HPLC and GC-MS. It is imperative that the TOC measurement be monitored for accuracy to prevent negative results.

TOC monitoring

TOC monitoring is a useful technology that provides a real-time measurement of the actual level of organics in the product water. Product water is regularly being sampled and tested for the level of organic impurities in the water at various intervals. To accomplish this, the conductivity (C1) of the product water is measured and the value stored in the water system's processor. During recirculation, the water is then sent through the system's UV bulb, where it is irradiated with UV light. This oxidises any organics present in the product water. The oxidation of the organics creates ions, which are then measured by a downstream conductivity cell (C2). The amount of extra ions in the water is directly proportionate to the amount of organics in the water, if the UV bulb is working properly. The difference between the conductivity cells is calculated and a TOC value is displayed.

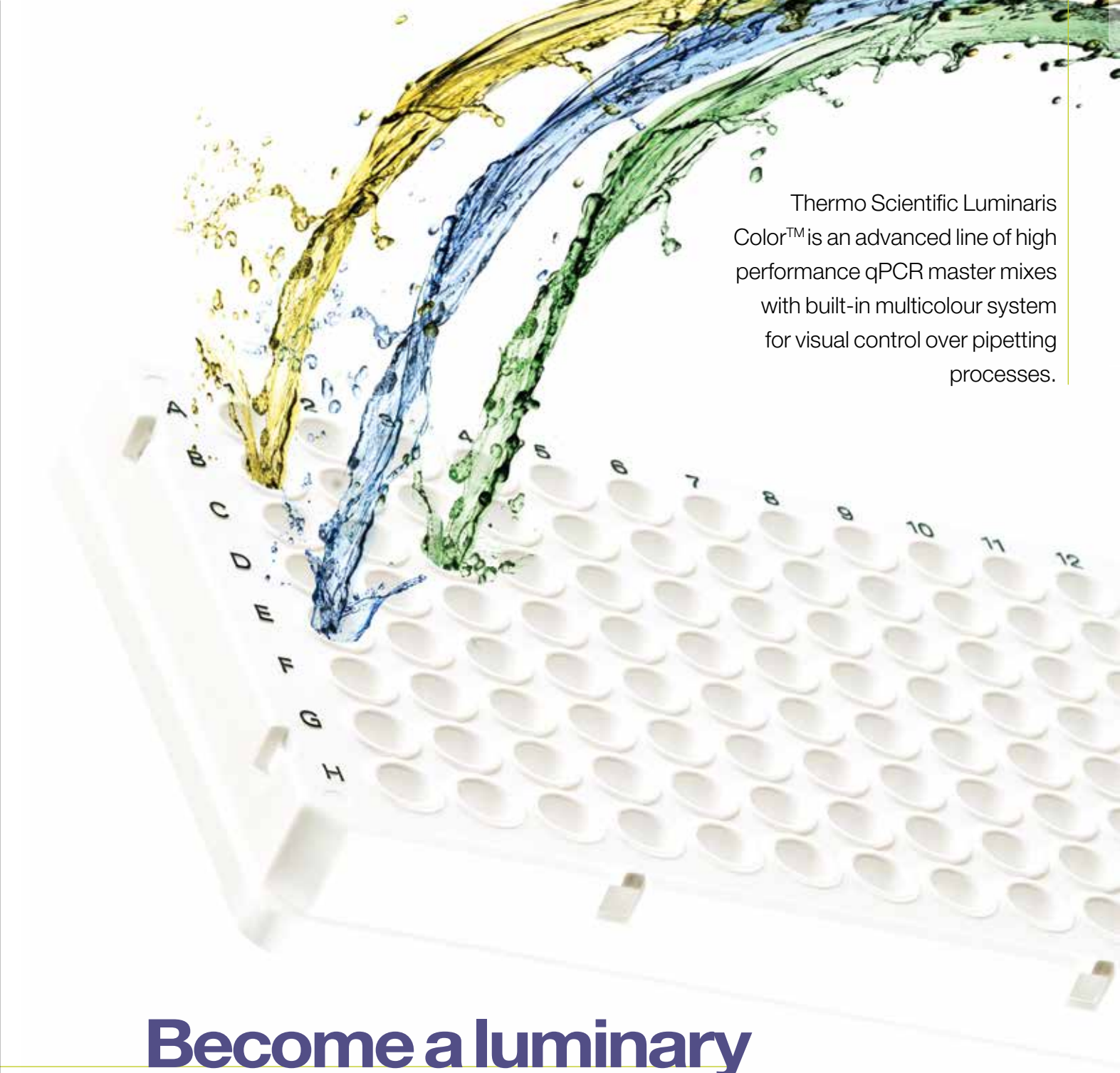
UV intensity monitoring – safeguarding TOC

The accuracy of the TOC measurement depends on how well the UV bulb irradiates the water. If the bulb is not fully illuminated, the total amount of organics in the water will not be oxidised, resulting in a false reading. To protect against this, Thermo Scientific engineers created a photo electrode that directly monitors the UV lamp, and ensures that it is working properly. If there is a problem, the system is designed to display an error.

Summary

If your application demands on extremely low levels of organics, UV intensity monitoring can help ensure that your TOC measurements are accurate.

Learn more at www.thermoscientific.com/**purewater**



Thermo Scientific Luminaris Color™ is an advanced line of high performance qPCR master mixes with built-in multicolour system for visual control over pipetting processes.

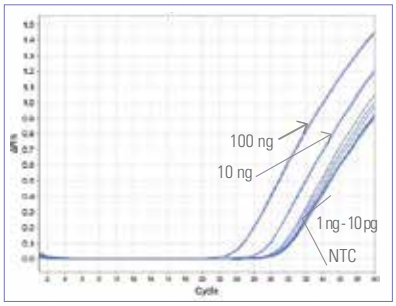
Become a luminary in your qPCR world

The Luminaris™ qPCR Master Mixes are specially formulated to produce the most consistent and reproducible qPCR data using probe or SYBR Green chemistry across all real-time PCR platforms. The Master Mixes contain Thermo Scientific Hot Start Taq DNA polymerase in an optimised reaction buffer for increased reaction efficiency, specificity and sensitivity. Uracil-DNA Glycosylase (UDG) included in the Master Mixes degrades the uracil-containing PCR products carried over from previous experiments.

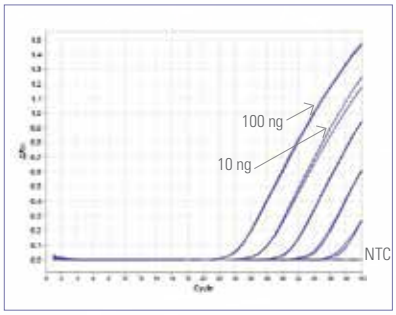
UDG for control over carry-over contamination

Due to the high sensitivity of qPCR, even minute amounts of contaminating DNA can lead to false positive results. The UDG enzyme included in Luminaris qPCR Master Mix degrades possible carryover amplicons from previous qPCR runs. This ensures amplification of only specific products from the sample and not from the environment.

DNA spike compromises qPCR results



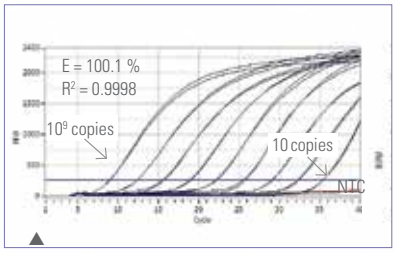
UDG removes DNA spike prior qPCR



UDG effectively removes carry-over contamination in qPCR. Amplification of *PGK-1* gene was performed from 100 ng - 10 pg of human genomic DNA spiked with dU-containing PCR product (3000 copies/sample). DNA was amplified with or without UDG treatment step on ABI StepOnePlus Real-time PCR instrument using Luminaris Color™ Probe High ROX qPCR MasterMix. NTC is the no-template control.

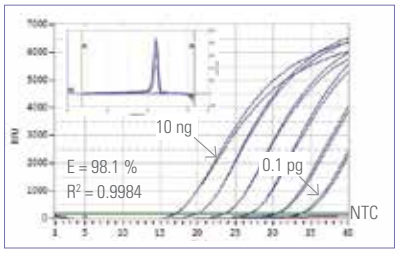
Stringent Hot Start chemistry and optimised buffer system for specificity, sensitivity and reproducibility

Luminaris Color™ qPCR Master Mixes are created to provide the highest specificity in qPCR. The chemically modified Hot Start Taq DNA polymerase in combination with the specially optimised reaction buffer considerably reduces non-specific amplification and formation of primer dimers. The highly reproducible qPCR results achieved from a wide range of input DNA amounts allow accurate quantification of genomic, plasma, viral and cDNA templates.

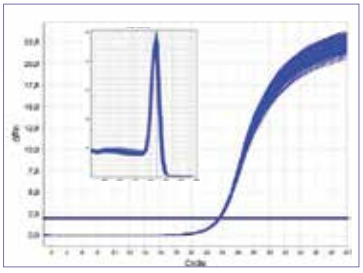


Wide dynamic range

10 to 10^9 copies of plasmid DNA were amplified using the Luminaris Color™ Probe qPCR Master Mix on the PikoReal 96 Real-Time PCR System. NTC is the no-template control.



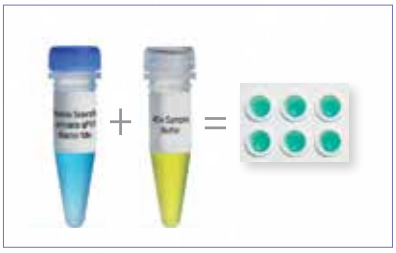
Human *c-Myc* gene was amplified with the Luminaris Color™ HiGreen qPCR Master Mix on the PikoReal 96 Real-Time PCR System using cDNA reverse transcribed from HeLa cells total RNA (10 ng – 0.1 pg) by Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Cat. #K1641). The insert shows the melting curve analysis results indicating high specificity of the amplification. Reaction efficiency E = 98.1%; R^2 = 0.9984



Human c-Myc gene was amplified in 96 individual reactions from 10 ng of human genomic DNA with the Luminaris Color™ HiGreen Low ROX qPCR Master Mix on the ABI 7500 Real-Time PCR instrument. Calculated CV = 0.24%.

Luminaris Color™ for control over pipetting process

Luminaris Color™ qPCR Master Mixes incorporate an inert blue dye, which does not affect the qPCR reaction, but significantly enhances the contrast between the reagent and plastic. The Yellow Sample Buffer complements the colour system by making the overall dispensing of qPCR reaction components quick and easy. This is particularly important for users of opaque white reaction plates and tubes, generally recognised as giving superior qPCR results.



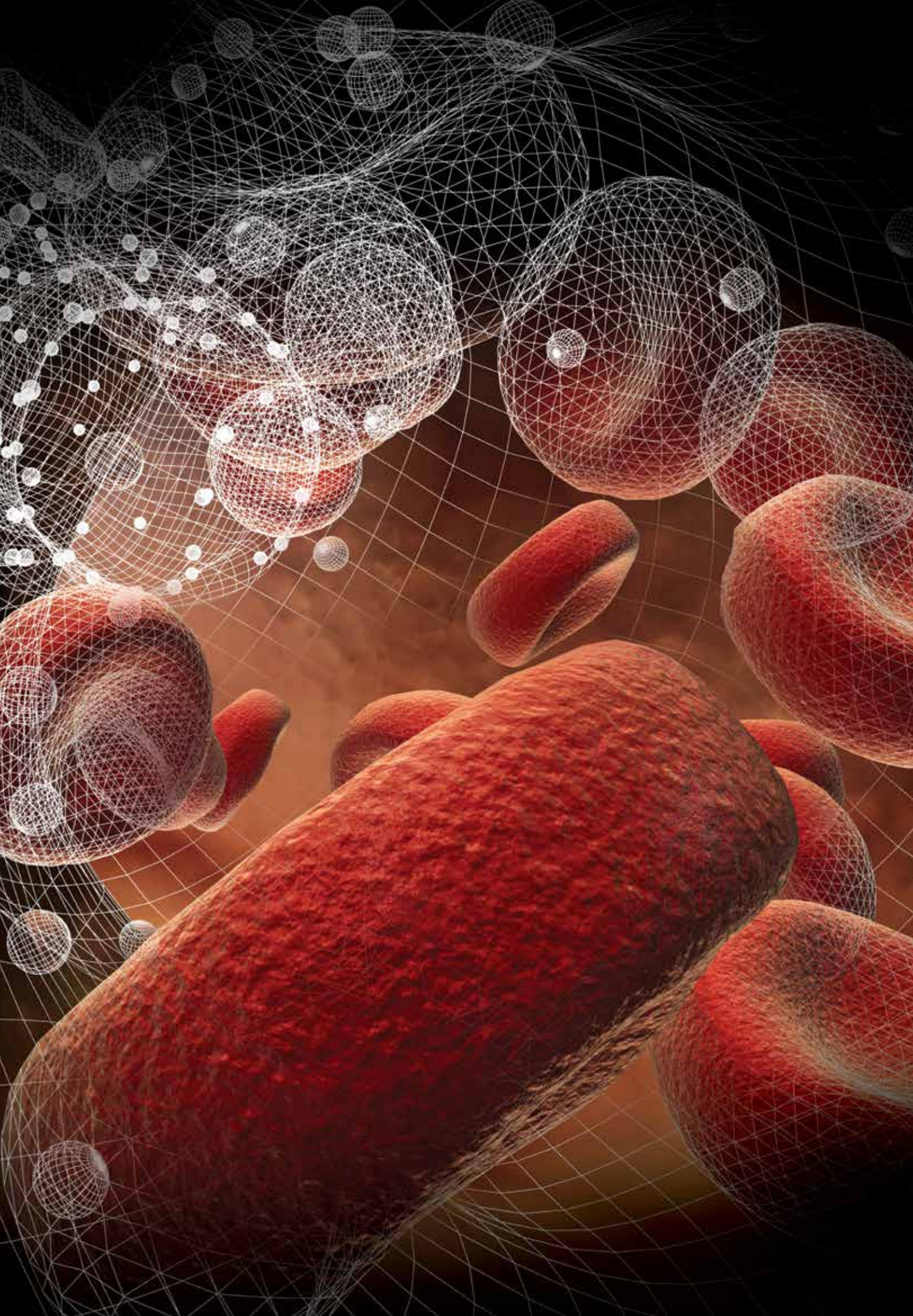
Luminaris Color™ qPCR Master Mixes (2X) contain a blue dye. The 40X Yellow Sample Buffer is included as a separate vial. This buffer can be mixed with the samples to provide visual aid when pipetting samples.

White reaction vessels are especially good for qPCR applications, as they deliver higher signal intensities than clear vessels. However, traditional colourless reaction components are poorly visible in white wells, making reaction setup more difficult. Luminaris Color™ qPCR Master Mixes overcome this difficulty and decrease the risk of pipetting errors. In the image, the blue wells contain only the 2X Master Mix. The green colour indicates the sample DNA with yellow colour has also been added into the reaction.

References

1. M. C. Longo *et al.*, Use of uracil DNA glycosylase to control carryover contamination in polymerase chain reactionsGene 93, 125-128 (1990).

driving bold progress



Accelerate. Advance. Achieve

The tools & technologies of p

TSU Ultra Low Temperature Freezer

Thermo Scientific TSU Series freezers deliver ultimate protection and optimum capacity for your most critical samples. The TSU Series achieve outstanding thermal performance, safety and security through state-of-the-art engineering. Packed full of features, the TSU Series has new Cabinet + Vacuum Insulation Panel Technology which increases the internal capacity of 2 inch vials over previous generation freezers—gaining up to 76% more capacity in the same footprint. The innovative, touch-screen user interface allows monitoring of the freezer's health 24/7 and access a detailed event log—simply touch the heart icon on the main screen to see freezer's temperature, access settings and operating parameters. Additional security options can be added with swip card user access to enable full users monitoring.

MyECL

The Thermo Scientific myECL Imager revolutionises image acquisition of protein and nucleic acid blots and gels detected via chemiluminescent, colorimetric or UV light-activated fluorescent substrates or stains. The compact benchtop instrument uses UV and visible light transillumination, specialised filters and advanced CCD camera technology to capture images with high sensitivity and dynamic range. The large, 10.4-inch touchscreen and on-board computer provide an elegant user-interface to program acquisition settings and manage (store and share) image files. Also included with the instrument is a five-computer license for the Thermo Scientific myImageAnalysis Software, a complete and powerful analysis tool.

Lynx Centrifuge

This superspeed centrifuge offers exceptional performance to meet the evolving application needs of academic and research facilities. With its versatile, high-throughput sample processing, built-in safety and efficiency and proven reliability, the Sorvall LYNX sets the standard for performance. Its 100,605 x g performance and maximised capacity up to 6 Liters for bottles, tubes or microplates. Plus breakthrough rotor innovations including Auto-Lock rotor exchange, Auto-ID instant rotor identification and lightweight and durable Thermo Scientific Fiberlite carbon fibre rotors that shorten run set-up time and increase rotor security.

NanoDrop Lite

The Thermo Scientific NanoDrop Lite Spectrophotometer is a compact, personal UV-Vis microvolume spectrophotometer that complements the full-featured NanoDrop 2000/2000c and NanoDrop 8000 instruments. The NanoDrop Lite provides rapid, accurate and reproducible microvolume measurements without the need for dilutions. It uses the same sample retention system that has become a hallmark of NanoDrop instruments; the sample is held in place by surface tension only. The NanoDrop Lite performs basic microvolume measurements. Its compact design, with built-in controls and software, make the NanoDrop Lite small enought to fit on any benchtop. The patented sample retention system allows samples to be pipetted directly onto the optical measurement surface. After measurement the sample is wiped off the measurement surface with a lint free lab wipe

Smarter Technolo

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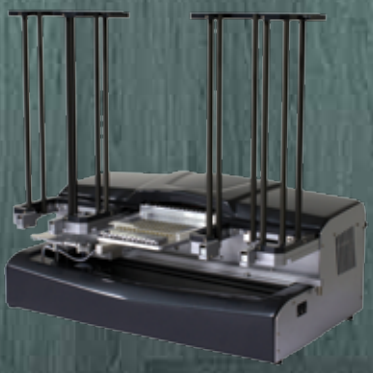
ClipTips

Thermo Scientific ClipTip pipette tips provide security with a unique and innovative interlocking technology that ensures a complete seal on every channel with minimal tip attachment and ejection force. Achieve newfound confidence knowing that once attached, your tips are locked firmly in place, and will not loosen or fall off regardless of application pressure. The innovative three interlocking clip design ensures the tip is held securely on the F1-ClipTip pipette until—and only until—it is released.



Formulatrix Tempest and Mantis Liquid Handling

Formulatrix liquid handlers provide robust, automated solutions to rapidly dispense reagents to meet experiment needs. The patent-pending microfluidic technology redefines liquid handling standards by replacing traditional methods with a microfluidic chip. This technology utilises independently controlled nozzles to dispense reagents into any well on a microplate. The compact design of the microfluidic chip has enabled the Mantis and Tempest to have a small footprint to save lab space.

Thermo Scientific Mass Spectrometric Immunoassay (MSIA) Tips

Thermo Scientific MSIA Tips are the next generation immunoaffinity approach that simplify peptide/protein enrichment for downstream quantification using mass spectrometers. Mass spectrometry has become an important tool for biomarker research because of its sensitivity, accuracy and capability of resolving post-translational modifications (PTMs). Many protein biomarkers in biological samples are present in low concentrations (picograms/mL) and it is necessary to concentrate and enrich them for downstream mass spectrometric analysis. Immuno-enrichment is commonly employed; however, most conventional methods are laborious and not able to deliver reproducible enrichment of biomarkers expressed in such low concentrations. MSIA tips provide a simple and effective way to enrich and concentrate target proteins down to femtomole level.

MagJet Extraction Kits

MagJET nucleic acid purification kits utilise magnetic bead-based technology allowing for highly efficient nucleic acid isolation from a variety of samples at any throughput. MagJET kits provide high-purity DNA and RNA ready to use in routine and demanding downstream applications. The proprietary high capacity paramagnetic particles are optimised to isolate nucleic acids with superior purity and yields compared to other kits on the market.

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perior Results

Complet

Filter Units - Now Stem Cell Tested

Thermo Scientific Nalgene Rapid-Flow disposable Filter Units with PES (polyethersulfone) membrane provide the last line of defense against cell culture contamination. Now you can guard your precious samples against contamination, and safely culture embryonic stem cells using filtered media - as long as you have the right filters and membranes! Our recent study (Culturing embryonic stem cells using media filtered with Thermo Scientific Nalgene Rapid-Flow PES filter units) shows that Embryonic Stem Cells grown in media filtered through Thermo Scientific Nalgene Rapid-Flow PES filters maintain normal growth and pluripotency, all without removing critical media components or adding deleterious compounds during the filtration process.

Tandem Mass Tags (TMT)

Thermo Scientific Tandem Mass Tag Kits and Reagents enable a rapid and cost-effective transition from method-development to high-throughput protein quantitation. The tags consist of TMT0 (zero), the TMTduplex and the TMTsixplex set. The TMT0 Label Reagent allows testing and optimisation of sample preparation, labelling, fractionation and MS fragmentation for peptide identification and reporter detection without using the more costly isotope-labelled compounds. The TMTduplex allows duplex protein profiling for small studies. The TMTsixplex allows sixplex protein profiling for multiple conditions, including time courses, dose responses, replicates or multiple sample comparisons. Each isobaric tag is based on the same chemical structure, eliminating the need to modify labelling conditions or HPLC separation conditions between experiments.

Luminaris qPCR Master Mixes

The Luminaris qPCR Master Mixes are specially formulated to produce the most consistent and reproducible qPCR data using probe or SYBR Green chemistry across all real-time PCR platforms. The Master Mixes contain Thermo Scientific Hot Start Taq DNA polymerase in an optimised reaction buffer for increased reaction efficiency, specificity and sensitivity. Uracil-DNA Glycosylase (UDG) included in the Master Mixes degrades the uracil-containing PCR products carried over from previous experiments.

Thermo Scientific Water

Thermo Scientific Water is a complete line of water purification technologies which includes solutions for your most critical and everyday application needs, from electrodeionisation to reverse osmosis and distillation. Our water purification portfolio features advanced ergonomics and technology, including remote dispensing, UV intensity monitoring, small footprints and flexible dispensing options to provide a configuration that best suits your lab. Many water systems can be easily upgraded to allow for additional capacity.

Choice and Convenience

te workflow solutions

Nicolet FTIR

The Thermo Scientific Nicolet iS50, featuring purpose-built accessories and integrated software, is an all-in-one materials analysis workstation designed to help solve analytical challenges with ease. The highly flexible system can be upgraded from a simple FT-IR bench to a fully-automated multi-spectral range system that can acquire spectra from the far-infrared to visible. Users can initiate novel ATR, Raman and NIR modules at the touch of a button, enabling access to these techniques without manually changing system components.

Phusion High-Fidelity DNA Polymerases

Incorporating a unique dsDNA-binding domain, Phusion DNA Polymerase amplifies DNA with accuracy and speed unattainable with standard PCR enzymes, even on the most difficult templates. Phusion has a High fidelity (error rate 4.4 x 10^{-7} in Phusion HF Buffer); the speed of the polymerase allows short extension times (15-30 s/kb); the robust reaction means minimal optimisation needed and it produces increased product yields.

Thermo Scientific Pierce Luciferase Reporter Assay

Thermo Fisher Scientific has developed a family of luciferase activity assays that use novel luciferase genes from *Cypridina* and *Gaussia* combined with the Red Firefly luciferase gene and the green shifted *Renilla* gene. The assay family provides both flash- and glow-type assays, which differ in the stability of luminescence signals and in sensitivity. Some of the assays utilise luciferases that are secreted into the culture media. This feature allows live monitoring of the reporter activity during cell growth, without a need for cell lysis. In addition, the assay family includes unique dual luciferase assays for multiplexing purposes. The dual assays include two luciferases: one for measuring the experimental luciferase activity and the other for measuring control activity for normalisation.

Conical Centrifuge Tubes

Premium, high quality conical tubes that are environmentally friendly, offer the highest cleanliness with a recyclable, plastic rack, and allow for increased traceability with the largest writing area on the market. The Thermo Scientific Nunc tubes are the highest speed rated tubes on the market with our 15ml tubes rated to 10,500xg and the 50ml tubes rated to 17,000xg. Thermo Scientific Nunc Labware Products are made from high purity resins, and molded using our state-of-the-art processes. Plastic labware is a safer alternative to glass without sacrificing accuracy.

Today's molecular biologist relies on the plasmid, a closed, circular, double stranded form of DNA that is propagated in bacteria.

Large Scale

Plasmid DNA Preparation

Hui Li, Department of Pharmacology and Molecular Toxicology, University of Massachusetts Medical Center, Worcester, MA

Many different protocols can produce plasmid DNA in large quantities that are sufficiently pure for general cloning, enzyme digestion, sequencing, cellular transfection, *in vitro* transcription/ translation, protein expression and other purposes. For instance, DNA can be isolated in cesium chloride gradients, but this method requires an ultracentrifuge and utilises ethidium bromide, which must be handled with care.[1] Alternatively, gravity or spin column kits effectively purify plasmid DNA in superspeed and microcentrifuges. This brief highlights Thermo Scientific Sorvall superspeed centrifuges and rotors that can be used in concert with the commercially available DNA preparation kits and also provides a low-cost method to obtain plasmid DNA in large-scale (about 1 mg) without using a kit.

Procedure

PROTOCOL 1: Cost-Efficient, Large-Scale DNA Plasmid Prep using only floor standing Superspeed Centrifuges from start to finish.

Hui Li, Department of Pharmacology and Molecular Toxicology, University of Massachusetts Medical Center, Worcester, MA.

This procedure describes the isolation of plasmid DNA using a floor model superspeed centrifuge from start-to-finish. The Thermo Scientific Sorvall LYNX 6000 superspeed centrifuge employs a diverse array of rotors and can accommodate a wide variety of applications.

1. Using the appropriate antibiotic selection, grow 500 mL or more of bacterial culture (usually overnight at 37 °C, with shaking).
2. Divide culture by pouring into 250 mL bottles. Pellet bacteria by performing centrifugation in a Sorvall® LYNX 6000 superspeed centrifuge with a Thermo Scientific Fiberlite carbon fiber rotor (such as the Fiberlite® F14-6x250y, F12-6x500 LEX, F10-4x1000 LEX, or F9-6x1000 LEX rotor) with the following parameters: 6,000 x g for 15 min at 4°C.
3. Decant supernatant and thoroughly resuspend bacteria in one of the bottles with 15 mL buffer A. Transfer bacteria to the other bottle and resuspend the combined pellets. There should be no "clumps" remaining.
4. Add 15 mL buffer B, mix by inversion 5-6 times and let sit at room temperature for 5 min. Solution should become more clear and viscous.
5. Add 15 mL buffer C, mix by inversion 5-6 times. A heavy precipitate will form; swirl to break up precipitate.
6. Centrifuge at 20,000 x g for 20 min at 4 °C.
7. Pour supernatant through cheese cloth and collect in a clean bottle. Note: An additional spin for 10 min may pellet any excessive debris not cleared in the first spin.
8. Add an equal amount of isopropanol to the bottle and swirl gently to mix. Perform centrifugation with the following parameters: 18,000 x g for 30 min at 4 °C. Note: Use a lab pen to mark the outer edge of the bottle before centrifugation to help locate the somewhat clear and translucent pellet (also applies to subsequent steps).
9. Carefully remove the supernatant. Add >50 mL of 70% ethanol to the 250 mL bottle and agitate to resuspend and wash the DNA. Perform centrifugation with the following parameters: 18,000 x g for 10 min at 4 °C.
10. Remove all the supernatant and dry the pellet in a vacuum dessicator for 30-60 min. Over-drying the pellet can prevent effective DNA resuspension in subsequent steps.
11. Dissolve the pellet in a total of 2 mL TE containing RNAase at a final concentration of 100 µg/mL. If dissolved in higher volumes, the DNA may be too dilute to effectively precipitate in subsequent steps.
12. Transfer to a 15 mL or similar volume tube and incubate at 37 °C for 1 hr.

13. Precipitate the DNA for 30 min on ice with 0.5 volume 20% PEG 8000, 2.5 M NaCl or with one volume of 13% PEG 8000, 1.6 M NaCl.
14. Perform centrifugation in the Thermo Scientific Fiberlite F14-14x50cy rotor with 15 mL conical adapters or in the Thermo Scientific A21-24x15c rotor with the following parameters: 20,000 x g for 20 min at 4 °C.
15. Carefully remove all the supernatant. The pellet may be very translucent and smeared along the outer edge of the tube. It is critical to remove all the PEG.
16. Dissolve the DNA in 700 µL of TE or 10 mM Tris and transfer to a high performance 1.5 mL micro-centrifuge tube.
17. Add an equal volume (700 µL) of phenol, vortex 30 sec and perform centrifugation in the Thermo Scientific Fiberlite F27-48x1.5 rotor with the following parameters: 19,000 x g for 5 min at 4 ºC.
18. Remove the upper aqueous phase and transfer to another high performance microtube containing 350 µL phenol and 350 µL chloroform.
19. Vortex and perform centrifugation with the following parameters: 19,000 x g for 5 min at 4 °C.
20. Perform a final extraction in 700 µL chloroform. Repeating the phenol/ chloroform extraction may increase DNA purity. A back extraction once with 700 µL of TE can increase yield.
21. Precipitate the DNA by the addition of 0.8 volume of isopropanol and 0.1 volume of 3M NaOAc, pH 4.5-5.5. Mix several times by inversion. Perform centrifugation in the Fiberlite F27-48x1.5 rotor with the following parameters: 19,000 x g for 15 min at 4 °C.
22. Decant supernatant and carefully wash the DNA pellet with 1 mL of 70% EtOH.
23. Air dry pellet and dissolve in 500-1000 µL Tris or TE.
24. Measure the OD260 and OD260/280 and calculate DNA concentration (DNA concentration in g/L = (OD260)(50)(Dilution)) and assess purity. Note: An OD260/280 below 1.8 suggests impurities. Double phenol/chloroform extraction can improve the ratio.

Table 1:

Solutions Needed
Buffer A: 50 mM Tris-HCl, pH 8.0; 10 mM EDTA
Buffer B: 0.2 M NaOH; 1% SDS
Buffer C: 3 M Potassium Acetate, pH 5.5
Isopropanol
70% Ethanol
RNAase A stock solution: 10 mg/mL
PEG/NaCl solution: 20% PEG 8000, 2.5 M NaCl or 13% PEG 8000, 1.6 M NaC
Phenol, Tris buffered to pH 8.0
Chloroform
10 mM Tris or TE
3 M Sodium acetate, pH 4.5-5.5

PROTOCOL 2: Using Superspeed Centrifuges for Plasmid DNA Preparation with Commercially Available Kits
Protocol 1 describes the process for plasmid DNA preparation without using a commercially available kit.

Many commercially available kits for plasmid DNA preparation that call for the use of a centrifuge with high speed and large volume capabilities, such as the Sorvall LYNX 6000 superspeed centrifuge. The initial steps in DNA preparation involves the pelleting of bacteria in which the plasmid of interest has been propagated. Individual bacterial cultures with volumes up to 6 L can be processed using the Sorvall LYNX 6000 superspeed centrifuge. Centrifugation at 6,000 x g for 10-15 min at 4 °C is sufficient to pellet bacteria grown in a 500 mL or larger culture. For smaller volume cultures, the time and speed for pelleting is reduced; centrifugation at 5,000 x g for 10 min at 4 °C is sufficient for a 150 mL bacterial culture. Table 2 lists a selection of rotors the Sorvall LYNX 6000 superspeed centrifuge that will serve to accommodate small and large scale pelleting (up to 6L) needs prior to DNA purification.

Table 2:

Thermo Scientific Rotor	Capacity (place x mL)	Max Speed (rpm)	Max RCF (x g)
Fixed Angle Rotors			
Fiberlite F9-6x1000 LEX	6 x 1000	9,000	17,568
Fiberlite F10-4x1000 LEX	4 x 1000	10,500	20,584
Fiberlite F12-6x500 LEX	6 x 500	12,000	24,471
Fiberlite F14-6x250	6 x 250	14,000	30,240
Swinging Bucket Rotors			
BIOFlex HC	4 x 1000	5,500	7,068
BIOFlex HC with adapter	4 x 500	5,500	7,068
BIOFlex HC with adapter	8 x 250	5,500	7,068
BIOFlex HS	4 x 400	7,000	10,025

Following the initial pelleting of bacterial culture, DNA purification steps require centrifugation at >15,000 x g. For ease of use and to ensure the integrity of samples, the use of sterile 15 mL to 50 mL conical tubes is desired, however, these conical tubes are typically limited to <7,000 x g. The unique Thermo Scientific conical tube rotors and conical tube adapters allow for the use of disposable sterile conical tubes at RCFs up to 63,409 x g. Table 3 lists a selection of rotors and adapters for the Sorvall LYNX 6000 superspeed centrifuge that will serve to accommodate the forces required by most commercial kits.

Table 3:

Thermo Scientific Rotor	Capacity (place x mL)	Max Speed (rpm)	Max RCF (x g)
Fixed Angle Rotors			
Fiberlite F14-14x50cy	14 x 50	14,000	33,746
Fiberlite F14-14x50cy with adapter	14 x 15	14,000	33,746
A21-24x15c	24 x 15	21,500	63,049
Swinging Bucket Rotors			
TH13-6x50 with adapter 75007322	6 x 50	13100	30,314

Conclusion

This application brief describes the wide variety of superspeed offerings provided by Thermo Fisher Scientific to accommodate the needs of molecular biologists during plasmid DNA preparation. Thermo Scientific equipment accommodates small and large volume bacterial cell pelleting and offers efficient, versatile, lightweight, and reliable rotors.

Table 1. Solutions for DNA preparation

Table 2. Rotors available for DNA preparation in the Sorvall LYNX 6000 superspeed centrifuge.

Table 3. High speed 15 mL and 50 mL conical tube rotors and adapters available for DNA preparation in the Sorvall LYNX 6000 superspeed centrifuge.

References

1. Noles, S.R., Schwartz, M.W., Fischer, R.A., Martin, R., Schneider, W., Jagadeeswaran, P., Rao, K.J. 2008. Traditional Methods for CsCl Isolation of Plasmid DNA by Ultracentrifugation.

Selecting Conical Tubes
for Centrifugation Applications

By Stephanie Carter, Wenxiao Lu, Marci Moore, Joe Granchelli & Cindy Neeley

Laboratory centrifuges are common, everyday instruments. While centrifuge usage is widespread, the selection of the proper centrifuge and rotor system, along with the proper centrifuge tube consumable, can often be a challenging experience.

There are several factors to consider when selecting the correct conical tube for centrifugation. These include the relative centrifugal force required, the fit of the particular tube in the rotor, sample volume, and the compatibility of the sample to the tube material.

Understanding the requirements of a centrifugation application before selecting a conical tube can prevent sample leakage or loss, allow for easy sample recovery, and reduce the risk for potential damage to the centrifuge and rotor.

Factors to consider when choosing a conical centrifuge tube

A. RPM vs. RCF

All centrifuge tubes have a maximum speed rating determined by the manufacturer. Vessels used at speeds higher than the recommended rating can fail, resulting in sample loss and potential damage to the centrifuge and rotor. Most protocols specify speed in either revolutions per minute (RPM) or relative centrifugal force (RCF). It is crucial to understand the difference between RPM and RCF. The rotor revolves at the specified RPM and the force applied to the contents is dependent on the rotor's radius, with larger radii applying greater force on the sample. RCF represents the gravitational force being applied to the sample. It determines the centrifugation outcome independent of rotor size. RCF is measured in force x gravity, or g-force, and is more relevant to the actual impact and outcome of the centrifugation than RPM. Users should verify the required RCF by their specific applications to ensure that the required centrifugation force does not exceed the manufacturer's specified g-force rating.

B. Fit of the conical tube in the centrifuge rotor

It is important to note that the centrifuge tube performance largely depends on how well the tube fits in the rotor (or rotor adapter). For optimal tube performance it is imperative that there is contact between the tube and the rotor/adapter so that centrifugal force can be distributed to the rotor rather than the tube. Forces exerted directly on the conical tube may cause stress lines, bulging, or cracking. For example, when using a swing-out bucket rotor in a centrifuge unit, the direction of the g-force centres on the conical bottom of the tube locking the tube in the centre position of the rotor. In some cases, this does not allow the lower portion of the tube to make contact with the rotor/adapter. The centrifugal forces on the side wall of the conical tube cannot be transferred to the rotor and may cause damage to the lower portion of the tube (Figure 1).

When using a fixed angle rotor in a centrifuge unit, the side of the tube makes contact with the rotor/adapter and most of the centrifugal force exerted on the tube can be transferred to the rotor (Figure 2). Consequently, the conical tube in a fixed angle rotor can be spun at much higher RCF in comparison to a swing-out bucket rotor.

C. Chemical compatibility

In addition to the maximum RCF, when evaluating centrifuge tubes it is important to make sure that the sample components will not harm the plastic tube material. Chemicals can affect the strength, flexibility, surface texture, colour, and shape of the plastic. Chemical resistance is influenced by temperature, duration and frequency of exposure, chemical concentration, and centrifugal force. Physical and chemical changes which may be caused by chemical exposure include:

- Absorption of solvents, resulting in softening or swelling of the plastic
- Stress-cracking of the plastic
- Permeation of solvent in the sample through the plastic
- Dissolution of polymer in the sample

The Nunc conical centrifuge tubes are made of premier quality polypropylene. Table 3 displays information on chemical compatibility of common reagents to polypropylene centrifuge tubes.

D. Sample volume

Before selecting a centrifuge tube size, the desired sample volume should be determined. The sample volume is limited to the available rotors in the laboratory and the protocol being used. Generally, a centrifuge tube should be filled at least 75%. In some cases, such as with ultracentrifuge tubes, it is required that tubes are completely filled to prevent failure. Using centrifuge tubes less than half filled to capacity can lead to high levels of material stress and can result in tube failure. If the available sample volume is smaller than the available rotor capacity, adapters are available for most rotors from the manufacturer (Table 1, Table 2). Adapters allow a smaller-volume tube to be used at the appropriate fill volume. This is essential when the recommended maximum RCF is going to be applied for centrifugation.

Figure 1.

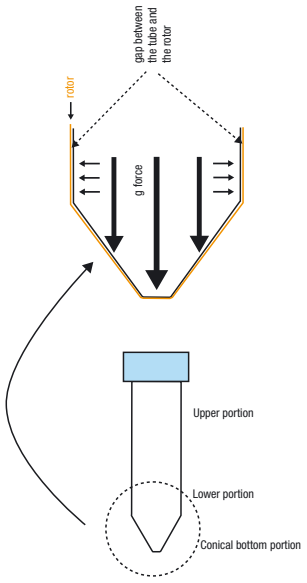

Figure 1. Swing-out bucket rotor and tube fit illustration. Centrifugal forces are exerted on the conical and lower portion of the tube. The imperfect fit of the lower portion of the tube to the rotor leaves a gap which may cause tube damage

Figure 2.

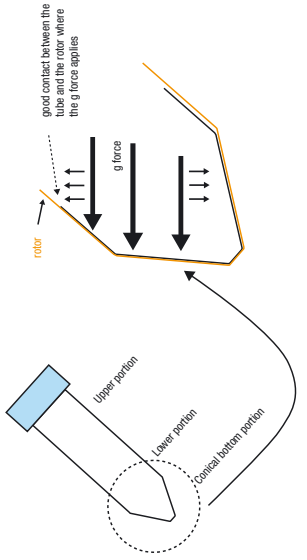

Figure 2. Fixed angle rotor and tube fit illustration. Centrifugal forces are exerted on the sides of the tube and transferred to the rotor/adapter.

Table 1. Recomended Maximum RCF for Thermo Scientific™ Nunc™ 15 mL Conical Tubes (Catalogue # 339650, 339651)

Thermo Scientific Centrifuge Model	Thermo Scientific Rotor Model	Thermo Scientific Adapter Catalogue #	Maximum RCF (x g)
Legend™ 1XR	BIOShield™ 720	75003678	3500
Legend 1XR	Fiberlite F15-6x100y	75003095	24652*
Legend 1XR	TX-400	75003682	4696*
Legend 1XR	TX-200	75003771	5580*
Multifuge™ X3R	TX-750	75003639	4816*
Multifuge X3R	BIOShield 1000A	75003642	5000
Multifuge X3R	Fiberlite F15-8x50cy	010-0378-06	24446*
Multifuge X3R	BIOLiner	75003673	2739*
Evolution™	Fiberlite F13-14x50cy	010-0378-06	15000

* Maximum speed for the centrifuge/rotor system is reached.

Table 1 and Table 2 display the recommended maximum RCF for the Thermo Scientific Nunc 15 mL and 50 mL conical tubes based on tests of 9 models of rotors in 3 types of centrifuge units. In most cases, a rotor adapter is needed to ensure good fitting of the tube to the rotor.

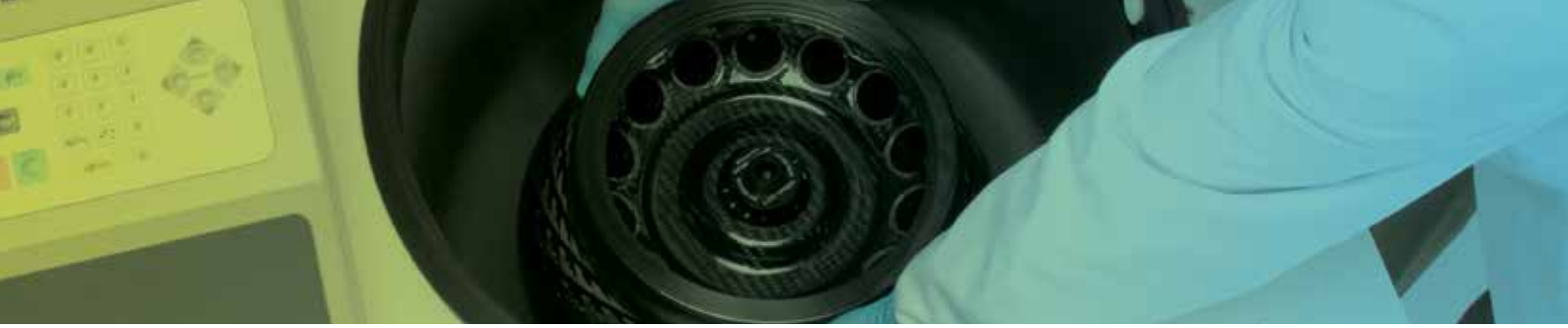

Table 2. Recommended Maximum RCF for Thermo Scientific Nunc 50 mL Conical Tubes (Catalogue # 339652, 339653)

Thermo Scientific Centrifuge Model	Thermo Scientific Rotor Model	Thermo Scientific Adapter Catalogue #	Maximum RCF (x g)
Legend 1XR	BIOShield 720	75003677	6500
Legend 1XR	Fiberlite F15-6x100y	75003103	23500
Legend 1XR	TX-400	75003683	4696*
Legend 1XR	TX-200	75003803	5580*
Multifuge X3R	TX-750	75003638	4816*
Multifuge X3R	BIOShield 1000A	75003643	6500
Multifuge X3R	Fiberlite F15-8x50cy	not necessary	20000
Multifuge X3R	BIOLiner	75003674	2739*
Evolution	Fiberlite F13-14x50cy	not necessary	15000

* Maximum speed for the centrifuge/rotor system is reached.

Summary

As in most endeavors, gathering the correct information ahead of time is essential. In this study, we demonstrated the important factors to consider when selecting the conical tubes for centrifugation so that sample leakage or loss is prevented. Choosing the correct centrifuge consumables also ensures proper execution of centrifugation, and reduces the risk for potential damage to the centrifuge and rotor.

The factors we discussed in this article include the RCF required by the protocol, the fit of the particular conical tube in the rotor, sample volume, and the chemical compatibility of the sample to the tube material. Users should verify the maximum RCF of the centrifuge tube to ensure that the required speed does not exceed the manufacturer's rating. Here, we recommended the maximum RCF of the Nunc 15 mL and 50 mL conical tubes in several Thermo Scientific centrifuge and rotor systems while taking these factors into consideration.

Key: Table 3.

S = Satisfactory
S1 = Satisfactory, may cause discolouration.
M = Marginal; may be satisfactory for use in a centrifuge, depending on length of exposure and speed. Testing under operating conditions is suggested before actual run.
U = Unsatisfactory; not recommended.

Table 3. Chemical Compatibility of Common Reagents to Polypropylene Centrifuge Tubes

Classification	Chemical	Rating
Alcohols	Butanol, pure	S
	Ethanol, 100%	S
	Isopropanol, 100%	S
	Methanol, 15%	S
	Methanol, 98% x-750	S1
	Propanol, 100%	S
Cryopreservation Agents	Dimethyl sulfoxide (DMSO), pure	S
	Glycerol	S
Detergents	Sodium Dodecyl Sulfate (SDS), pure	S
	Triton X-100, pure	S
	Tween-20	S
Fixatives	Acetone, 50%	S
	Acetone, pure	M
	Formaldehyde, 10%	S1
	Formaldehyde, 30%	M
	Formalin, 10%	S1
	Formalin, 30%	M
	Gluteraldehyde, pure	S
	Paraformaldehyde, pure	M
Other	Beta- Mercaptoethanol, pure	M
	Cell culture media and sera	S
	EDTA, pure	S
	RNAzol	S
	Trypsin	S
	Toluene, pure	U
	Xylene, pure	U

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Improving productivity through compact automation

Many modern analytical laboratories face increasing workloads from a broad range of sample types with a simultaneous drive for faster results and more complex sample characterisation needs.

Many routine QC/QA laboratories can perform material analyses with single range, basic Fourier transform-infrared (FT-IR) instrument configurations. However, modern analytical laboratories face increasing workloads from a broad range of sample types with a simultaneous drive for faster results and more complex sample characterisation needs. Flexibility to analyse multiple sample types becomes mandatory when rapidly responding to these different application requests. Such diversity requires laboratory instruments to be reconfigured for specific measurements multiple times per day, taking time away from other critical activities. This also implies that laboratory personnel possess the necessary skills and experience to make decisions on how best to configure the instrument for a given application. In addition, frequent handling of delicate optics components presents a costly risk for instrument failure. As a result, many industrial laboratories choose to outsource complex analyses. These limitations inevitably slow the laboratory's ability to respond to urgent business needs.

The Thermo Scientific™ Nicolet™ iS™50 FT-IR spectrometer alleviates many of these productivity concerns by automating setup of the FT-IR system for multi-spectral range experiments (>20,000 cm^{-1} to 80 cm^{-1}) and for integrating techniques like FT-Raman, near-IR and mid/far-IR attenuated total reflectance (ATR) into a single workflow. Intelligent design behind the Nicolet iS50 spectrometer permits unattended, risk-free operation, increasing lab efficiency, sample throughput, and operational consistency between users. This capability is delivered in an economical, compact system (63 cm of linear bench space) enabling any laboratory to employ multiple techniques for their analysis.

Flexibility and Value-added Activities

Working labs need analytical flexibility to respond to a variety of situations where answers are critical for decision-making. Examples include deformulating mixtures to build a case for patent infringement, identifying counterfeit materials for product safety alerts, analysing forensic samples for criminal investigations, performing failure analysis to minimise production run delays, assessing process scale-up options for a new product launch, or troubleshooting customer complaints. Such diversity of applications requires the selection and installation of the correct instrument accessory as well as choosing the optimal source, beamsplitter, detector, optical path, and experimental conditions. Manually changing components and sampling parameters requires skill and may risk exposure of expensive optics to the external environment (i.e., dust, fingerprints or water vapor). In addition, changing these parameters can result in extensive wait times to equilibrate the instrument before the next measurement. These manual reconfigurations provide little added value to the laboratory workflow. Users must plan and set up batch experiments to minimise the number of steps. This creates bottlenecks, limiting access to the full capability of the instrument. As a result, labs are less able to address "emergency situations" without interrupting the batch run and resetting the instrument parameters. For instance, analysis of a polymer with additives requires mid-IR and far-IR plus Raman spectroscopy. This would entail three beamsplitter changes with associated risks in handling expensive components and instrument recovery times between changes.

The productivity improvements with the Nicolet iS50 FT-IR spectrometer come from two main sources. First, the internally mounted iS50 ABX Automated Beamsplitter Exchanger uses one-button simplicity (described as a Touch Point) to perform instrument setup and operation, providing a "one touch and done" workflow. The removal of manual handling and exposure of the optics to the environment means instant readiness. Second, the user need no longer care about which optics are installed. As seen in Table 1, the potential for error in manual operations is apparent when the array of possible component combinations is considered. With the Nicolet iS50 spectrometer, however, a user simply presses the Touch Point on the instrument to automate the configuration and ready the instrument for the experiment. For example, pressing the Touch Point on the iS50 NIR module automates the setup without requiring any understanding of which optics are used. What matters is performing NIR analysis – not worrying about

References

1. Heavy atoms or groups of atoms shift the IR wavenumber value lower, according to the relationship where $\tilde{\nu}$ is the IR wavenumber (cm^{-1}) and μ is the reduced mass. As the mass (μ) increases, the IR peak shifts to lower wavenumbers. $\tilde{\nu} \alpha \sqrt{1/\mu}$

Glossary

CaF_2 – calcium fluoride
DLaTGS – deuterated L-alanine doped triglycene sulphate
InGaAs – Indium gallium arsenide
KBr – potassium bromide

choosing the right components. The instrument takes care of this step.

Integration of the spectrometer with its modules and components allows the user to expand capabilities, increasing productivity with tools such as:

- Up to three detectors (such as near-, mid- and far-IR)
- The iS50 Raman sample compartment module
- The built-in diamond iS50 ATR sampling station
- The iS50 NIR module with integrating sphere or fibre optics
- The iS50 GC-IR module
- A sample compartment thermal gravimetric analysis-IR (TGA-IR Interface)

Figure 1 describes the analytical power the user can achieve with the iS50 spectrometer to obtain answers needed for time-sensitive decisions. With a single user interaction, the instrument can perform multiple measurements and analyses, resulting in a final report, even when unattended. The Thermo Scientific OMNIC™ software provides a user-friendly interface to set up applications quickly and generate spectra for definitive answers. By adding powerful analytical tools like the Thermo Scientific OMNIC Specta™ software with a library of over 30,000 spectra and multi-component searching (or the TQ Analyst™ software for chemometrics), a complete analytical workflow from sampling to results can often be achieved in less than 60 seconds.

This paper will demonstrate how the integration and automation of the Nicolet iS50 spectrometer leads to new levels of productivity, while minimising risk to costly components. Unlike most spectrometers, operating the Nicolet iS50 instrument becomes simpler as modules are added and as more manual steps are removed even when unattended.

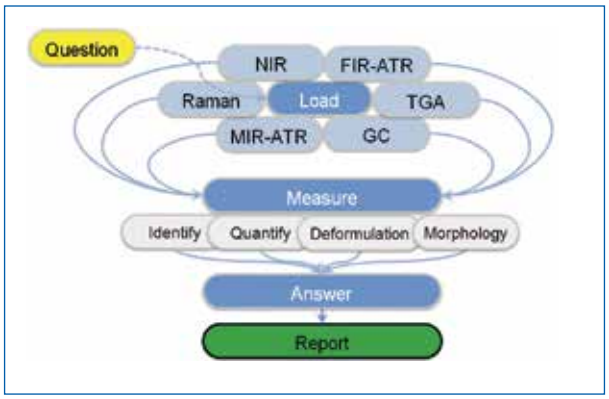


Figure 1: Nicolet iS50 analysis work

Automated Multi-spectral Analysis: Mid- and Far-IR ATR plus Near-IR

Most FT-IR users understand the utility of the mid-IR spectral range for qualitative and quantitative analyses. Less well known, the far-IR region can provide new and unique information. Simply put, as the mass of atoms involved in vibrations increases, the wavenumber decreases.[1]
Thus, for materials like organometallics or metal oxides, the IR absorption shifts below 400 cm^{-1} and below the range of standard KBr optics. Numerous polymers, sugars, and other large molecules also have far-IR information which may be useful or definitive to the analyst. Traditionally, collecting FT-IR spectra in both the mid-IR and far-IR region entailed significant sample preparation. This included changing hygroscopic optics and multiple detectors, and risking altered system performance from water vapor. The Nicolet iS50 spectrometer enables rapid analysis over the full mid-IR and well into the far-IR region (4,000 cm^{-1} to 80 cm^{-1}) when equipped with the iS50 ABX, iS50 ATR, and the correct beamsplitters.
The typical, multi-range FT-IR application requires opening the spectrometer to swap beamsplitters. This requires care in handling costly components and exposes the internal optics to the environment by disrupting purge or desiccation. This activity adds a recovery period to re-equilibrate the instrument before quality data can be collected. These wait times add

Table 1: Experiments made possible with the Nicolet iS50 FT-IR Spectrometer

Experiment	Source	Beamsplitter	Detector	Accessory
Mid-IR Transmission	Thermo Scientific Polaris™	KBr	KBr-DLaTGS	Standard Cells
Far-IR Transmission	Polaris	Solid Substrate	Polyethylene DLaTGS	Cells w/Far-IR Windows
Near-IR Transmission	White Light	CaF_2	InGaAs	Cuvettes
Mid-IR ATR	Polaris	KBr	Dedicated DLaTGS	iS50 ATR
Far-IR ATR	Polaris	Solid Substrate	Dedicated DLaTGS	iS50 ATR
FT-Raman	Raman Laser	CaF_2	Raman InGaAs	iS50 Raman

no value to operations, wasting the analyst's precious time. Integration and automation on the spectrometer eliminate non-productive wait times, improving efficiency.
As an example, Table 2 compares the steps needed to perform a full spectral analysis from far-IR to near-IR between the manual method (Typical) and the Nicolet iS50 method with built-in iS50 ATR and iS50 NIR module. This represents three spectral ranges in one sampling operation, a unique power of the instrument. Most important the built-in iS50 ATR optics and detector permit spectral data collection in both the mid- and far-IR regions. The analysis time decreases from around 30 minutes to less than seven.

With the Nicolet iS50 spectrometer, the user is able to load two sampling locations (the built-in ATR and the Integrating Sphere module), start the macro and walk away, while in the manual operation, continuous attention is needed to swap the beamsplitters at the right moments.

This seemingly hidden improvement allows unattended operation, permitting productivity through automation. Figure 2 shows just the mid- and far-IR spectra collected from acetylferrocene analysed using an OMNIC macro-controlled workflow. The additional information from the far-IR spectra is clear – the low end triplet verifies that the iron is sandwiched between the cyclopentadiene rings. The NIR data is not shown, but the entire process required seven minutes, including collection of the mid- and far-IR backgrounds. Automation also reduced the total hands-on time of the user (pressing buttons, loading sample) to ≈20 seconds.

Multiple Techniques and Multi-range Analysis: Enhanced Flexibility

The Nicolet iS50 spectrometer can be configured with FT-Raman, NIR, and wide-range diamond ATR. Switching between these experiments raises concerns of instrument recovery time (purge), exposure/handling of optics, and potential confusion or user error. The experiments are often seen as independent activities for these reasons. The spectrometer with iS50 ABX simplifies this apparently complex situation to one step – initiation of a macro. The Nicolet iS50 instrument shown in Figure 3 is configured with the iS50 NIR, iS50 Raman, iS50 ATR and the iS50 ABX modules and shows how easy sample loading and analysis can be done.
For operating one module at a time, the user need only press the associated Touch Point. Seen more closely in Figure 4, Touch Points make one-button operation effortless when switching between modules (sampling stations) and automating optics exchange. Rather than thinking through the components needed (light source, beamsplitter, optical path and detector) to run an experiment, the user simply presses the Touch Point to switch from an ATR to an NIR measurement and waits until the instrument indicates that it is ready to begin. This error-free operation is done in 30 seconds. The Nicolet iS50 analytical power in Figure 1 becomes clear when the four data collections – mid-IR and far-IR ATR, NIR, and Raman – are performed in one workflow.

Collecting spectra from each of these modules using a conventional manual approach required about 50 minutes, including sample loading, optical changes, time for equilibration, and optimisation of the Raman signal. The analyst needed to be present throughout the experiment to perform the beamsplitter changes and collect various backgrounds for each sampling station. At the end of the 50 minutes, four spectra and their analyses were available. Actual data collection took 5 minutes and total hands-on time was 45 minutes, representing inefficient use of the analyst's time.
In contrast, the results shown in Figure 5 emerged from a single OMNIC-macro operation. The macro was programmed to begin by collecting backgrounds for the mid- and far-IR ATR, and then switched to the iS50 Raman module. Next the samples were

Table 2: Far-infrared analysis: Typical versus Nicolet iS50 process *With the iS50 ATR present, the far-IR background (BKG) is collected, the iS50 ABX swaps beamsplitters, and the mid-IR background is collected in <1.5 minutes. The sample is loaded and the spectra are collected in sequence. All times are approximate.

Process Step	Typical	Time (minutes)	Nicolet iS50 with Built-in ATR	Time (minutes)
Sample Preparation	Grind, Mix	10	**None**	0
Mid-IR Background	Collect BKG	0.5	Collect BKG (2nd)*	1.
Mid-IR Collect	Load Sample, Collect Spectrum	2	Load Sample, Collect Spectrum	1
Change Optics	Manual Exchange	0.5	Automated	0.5
Recovery Time	Wait for Purge	5–10	**No Recovery Time**	0
Far-IR Background	Collect BKG	0.5	Collect BKG (1st)*	0.5
Far-IR Collect	Load Sample, Collect Spectrum	2	Load Sample, Collect Spectrum	1
Change Optics (NIR)	Manual Exchange	0.5	Automated	0.5
Recovery Time	Wait for Purge	5	**No Recovery Time**	0
Collect Background	Collect BKG	0.5	Collect BKG	0.5
Collect Sample	Load Sample, Collect SAM	1	Collect SAM	0.5
Data Analysis (Search)	Perform Search	2	Automated Search	0.5
Total Time		**29.5–34.5**		**6.5**

loaded on the ATR, NIR, and Raman sampling stations. After optimising the signal using the autofocus feature of the Raman module, the macro was initiated, and the analyst walked away. From starting the macro to completion of the final report, the analysis took less than 12 minutes, representing a time savings of over 70%. The actual data collection time was again 5 minutes, however, total hands-on time for the analyst was only 2 minutes – a highly efficient use of the analyst's (and the instrument's) time.

Conclusion

Many forces contribute to new pressures on industrial analytical laboratories: increased sample loads, decreased staffing, retirement of experts, and shrinking budgets. The Thermo Scientific Nicolet iS50 FT-IR spectrometer makes a significant contribution to alleviating these challenges through automation in a multi-tasking, single platform instrument. The Nicolet iS50 spectrometer greatly simplifies and streamlines workflows by decreasing the number of steps with one-button ease and macro operations performed by the analyst. In addition, risks inherent in manual operations (e.g., user error, environmental exposure) and long recovery times are eliminated. Analysts of any skill level can successfully obtain meaningful results with minimal hands-on time.

Technology designed to improve workflow can be found in the iS50 ABX and task-specific modules (i.e., Raman, NIR, TGA-IR etc.). The Touch Point operation simplifies access to the full range of capabilities by automatically configuring the optics (near-, mid- and far-IR) and switching between sampling stations (modules) in seconds for enhanced productivity. For the modern industrial lab, the Nicolet iS50 FT-IR spectrometer offers a powerful new tool that goes beyond routine FT-IR to more comprehensive analyses (e.g., FT-Raman and far-IR), adding value to laboratory activities in a compact, easy-to-operate platform.

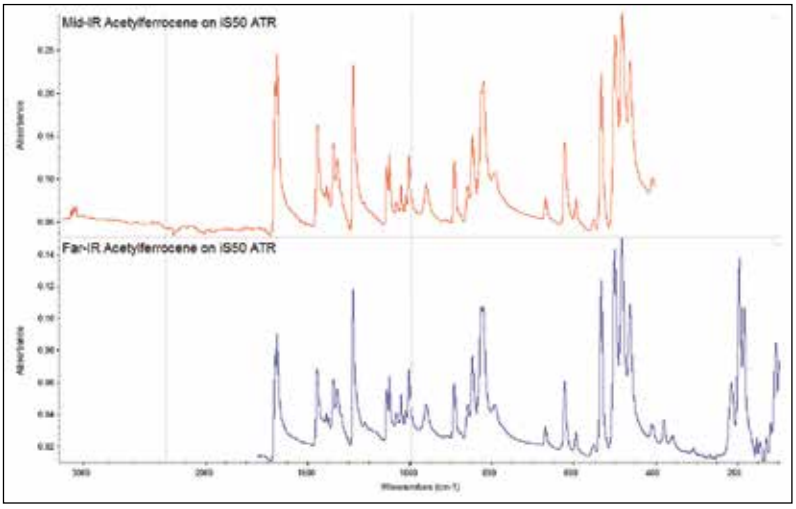
Figure 2: Mid-IR and far-IR spectra of Acetylferrocene. The far-IR optics permit collection to 1700 cm^{-1}, which may be sufficient (fingerprint and far-IR) for many applications.

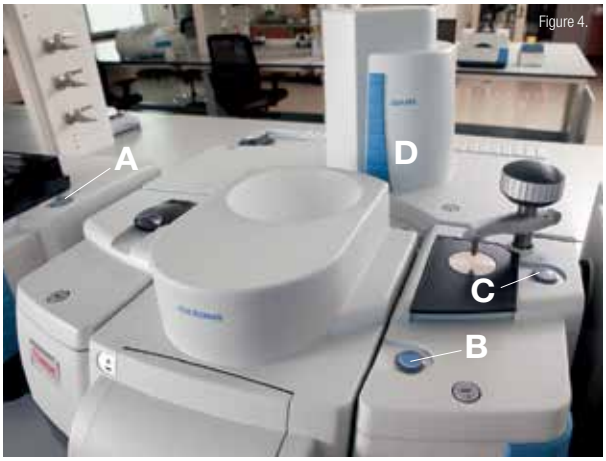
Figure 3: The Thermo Scientific Nicolet iS50 FT-IR spectrometer configured for FT-Raman, near-IR, and mid/far-IR ATR with the automated beamsplitter exchanger.

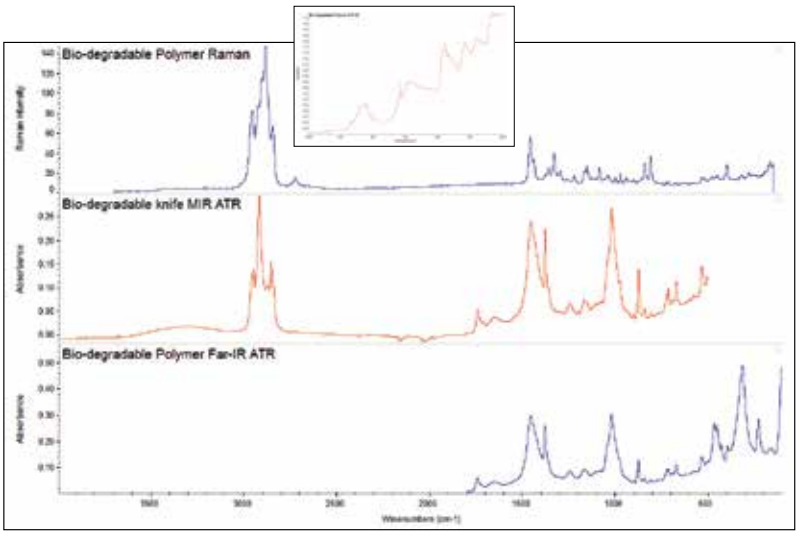
Figure 4: Touch Points on the Nicolet iS50 spectrometer employ one-button switching between modules and the iS50 ABX automates optics set-up

Touch Point A – NIR module
Touch Point B – Raman module
Touch Point C – Built-in diamond ATR
Component D – ABX Automated Beamsplitter Exchanger

Figure 5: Multi-technique data for a recyclable plastic component using the spectrometer pictured in Figure 3. Inset shows NIR independently for clarity.







Immunoaffinity capture and enrichment of protein and peptide targets is a widely utilised method for sample cleanup prior to mass spectrometric analysis. Termed the Mass Spectrometric Immunoassay (MSIA) [1], this hybridised combination of micro-scale purification with highly sensitive detection has repeatedly demonstrated the ability to detect much lower abundance proteins from a given proteome as compared to other fractionation methods[2,3].

A Universal Mass Spectrometric Immunoassay (MSIA™) Model system

based on human insulin-like growth factor 1 (IGF1)

Eric E. Niederkofler, Senior Scientist, David A. Phillips, Technician, Dobrin Nedelkov, Manager of Research and Development and Urban A. Kiernan, Senior Scientist, Thermo Fisher Scientific, Tempe, Arizona. Bryan Krastins, Senior Applications Scientist, Scott Peterman, Senior Applications Scientist, Alejandra Garces, Senior Applications Scientist and Mary F. Lopez, BRIMS Center Director, BRIMS Center, Thermo Fisher Scientific, Cambridge, Massachusetts.

Figure 1. Protein A/G MSIA-Tip Workflow: Protein A/G MSIA-Tips are used by repetitive pipetting of solutions, allowing for efficient interaction between capture and analyte reagents within the porous monolithic solid supports. First, antibody is loaded onto the Protein A/G surface. The loaded antibody is then used to immuno-capture and enrich its targeted analyte from biological samples. After rinsing, retained analyte is eluted and subjected to reduction, alkylation and standard tryptic digestion and LC-MS/MS protocols.

Figure 2. An 8-point IGF1 calibration curve was generated from standard samples consisting of 1, 5, 10, 25, 100, 500, 1000, and 1500 ng/mL of IGF1 and 500 ng/mL of the internal standard (LR3-IGF1). MS area ratios of IGF1:LR3-IGF1 are plotted against the IGF1 concentrations and used to calculate the concentrations of IGF1 in unknown samples based on the samples' area ratios. Replicate analyses of a single plasma donor demonstrated CV of 8.5%.

Even though this approach to sample purification is now the staple in proteomics methods; more robust, consistent, and versatile methods for performing this sample purification and enrichment step are a growing necessity. To further improve upon this front-end sample processing, a new technology, Thermo Scientific™ MSIA™, was tested. In the form of a functional pipettor tip, their performance was evaluated (see Application Notes: MSIA1002 and MSIA1003) and bench marked against other technologies. However, to perform these tests, a model system translatable to various ligand surfaces including Protein A, Protein G and Protein A/G was needed. Presented are the results of a developed model system for the Protein A/G MSIA-Tips based on Insulin-Like Growth Factor 1(IGF1), a clinically relevant endocrine and oncology protein biomarker [4,5,6].

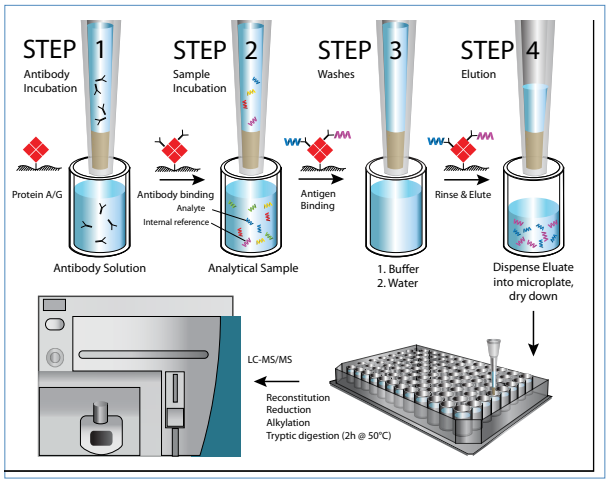
Materials

- Thermo Scientific Protein A/G MSIA-Tips
- Thermo Scientific Versette Liquid Handling Platform
- Anti-human IGF1 antibody
- Human recombinant IGF1 (IGF1 standard)
- Recombinant LR3-IGF1 (Internal reference standard)
- Thermo Scientific Pierce BupH Phosphate Buffered Saline (PBS)
- Antibody dilution buffer - 10mM MES/0.1% polysorbate 20, pH 5
- EDTA plasma, human donor
- Trypsin
- LC-MS grade water
- Thermo Scientific Optima grade Formic Acid (FA)
- Thermo Scientific Optima grade Acetonitrile (ACN)
- Standards dilution buffer - 10 g/L BSA in PBS pH 7.2
- Sample dilution buffer - PBS/0.3% SDS
- Elution buffer - 33% acetonitrile/0.4% trifluoroacetic acid
- Reduction buffer - 10mM DTT in 30% isoproponal/0.1M ammonium bicarbonate pH 8.0
- Alkylation reagent - 0.5M Iodoacetamide/0.1M ammonium bicarbonate pH 8.5
- Thermo Scientific TSQ Vantage™ Triple Stage Quadrupole Mass Spectrometer
- Thermo Scientific Hypersil GOLD™ C18 column (50 mm x 2.1 mm, 1.9 µm particle size)

Methods

Samples: An 8-point IGF1 calibration curve was prepared by serial dilutions of recombinant human IGF1 into standards dilution buffer (concentration range 1-1500 ng/mL IGF1). Replicate samples (n = 12) from a single EDTA plasma donor and IGF1 calibration curve samples were prepared by diluting 40 µL plasma/IGF1 standards with 20 µL 0.5 mg/L internal reference standard (in standards dilution buffer) followed by 100 µL PBS/0.3%SDS and incubated at room temperature for 30 minutes prior to extraction and enrichment with Protein A/G MSIA-Tips. Antibody Loading of Protein A/G MSIA-Tips: Protein A/G MSIA-Tips were loaded with 100 µL rabbit anti-human IGF1 antibody (0.01 mg/mL) following protocols provided in the user manual (total processing time 30 minutes). IGF1 Extraction and Enrichment: Co-extraction and enrichment of IGF1 and LR3-IGF1 were performed using a single Protein A/G MSIA-Tip (loaded with antibody) per each sample following the protocols provided in the user manual (total processing time 30 minutes). After extraction, IGF1 and LR3-IGF1 were digested and analysed by SRM as described below.

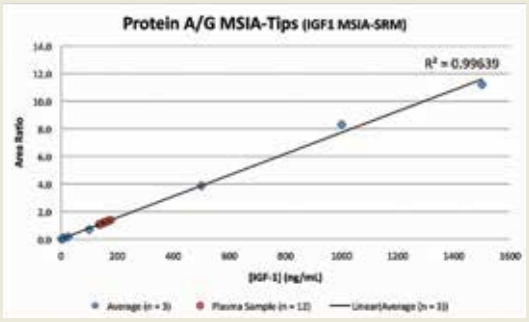
Figure 1:



Using the Versette liquid handler equipped with these tips, up to 96 samples were able to be processed in parallel with minimal user interaction.

The resulting IGF1 MSIA-SRM, using Protein A/G MSIA-Tips, demonstrated a wide linear dynamic range (Figure 2) and the ability to reproducibly quantify unknown IGF1 concentrations from plasma samples (Table A). Loaded with IGF1 antibody, the Protein A/G MSIA-Tips enabled for the efficient capture and enrichment of femtomole amounts IGF1 (Table A) with no additional sample preparation or depletion.

Figure 2:



LLOD	Assay Range	IGF1	Reproducibility, % CV (n = 12)
1 ng/mL (5.2 femtomole)*	1 - 1500 ng/mL (5.2 - 7800 femtomole)*	114 %	8.5 %

*Amounts based on a 40µL plasma sample volume.
Table A. Protein A/G MSIA-Tips IGF1-SRM assay characteristics.

Conclusion

We described the development of a novel model system, targeting human IGF1, to test the performance of Protein A/G MSIA-Tips. Using this test system, the MSIA-Tips demonstrated the ability to reproducibly perform quantitative measurements using SRM detection. These results clearly show how these devices can provided a mechanism for the highly specific and reproducible enrichment of a target analyte from plasma. Not only does this described SRM assay provide a uniform approach to technology evaluation, it also serves as a template for the development of future LC-MS/MS based MSIA methods.

Sample Elution and Trypsin Digestion: Captured IGF1 and LR3-IGF1 were co-eluted from the Protein A/G MSIA-Tips by repeatedly mixing (20 cycles of aspirating and dispensing 30 µL volumes) 50 µL of elution buffer within the wells of a 96-well plate using the MSIA-Tips with captured IGF1 and LR3-IGF1, thus eluting IGF1 and LR3-IGF1 into the 50 µL elution buffer within each well. Samples were lyophilised to dryness and then re-suspended in 30 µL of reduction buffer and allowed to reduce for 30 minutes at 37°C. Reduced samples were then alkylated by adding 2.4 µL alkylation reagent and incubating in the dark at room temperature for 30 minutes. Reduced and alkylated samples were diluted with 92.5 µL of warm (50°C) 0.1M $NH4HCO_3$/5mM $CaCl_2$ and then digested by adding 25 µL 4 mg/L trypsin to each sample. Samples were allowed to digest for 2 hours at 50°C and then stopped by adding 5.3 µL of acid solution (3 µL 100% formic acid and 2.3 µL 1mg/mL glucagon). Injection volumes of 155 µL of the digests were injected into the LC-MS for SRM.

SRM Methods: SRM methods were developed on a Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer with a Thermo Scientific Accela™ pump, a CTC PAL® auto-sampler, and a Thermo Scientific Ion Max source equipped with a high-flow metal needle. A mass window of full width at half maximum of 0.7 (unit resolution) was used in the SRM assays because immuno-enriched samples have a very high signal-to-noise ratio. Reversed-phase separations were carried out on a Hypersil GOLD™ C18 column (50 mm x 2.1 mm, 1.9 µm particle size) with a flow rate of 240 µL/minute. Solvent A was 0.2% formic acid, and solvent B was 0.2% formic acid in acetonitrile.

Results and Discussion

A model system for the Protein A/G MSIA-Tips, based on Insulin-Like Growth Factor 1(IGF1), was developed to serve as a template for future LC-MS/MS methods that perform quantitative immuno-affinity proteomics. The Protein A/G MSIA-Tip workflow (Figure 1) proved to be simple and fast, requiring as little as 1 hour to provide immuno-purified IGF1.

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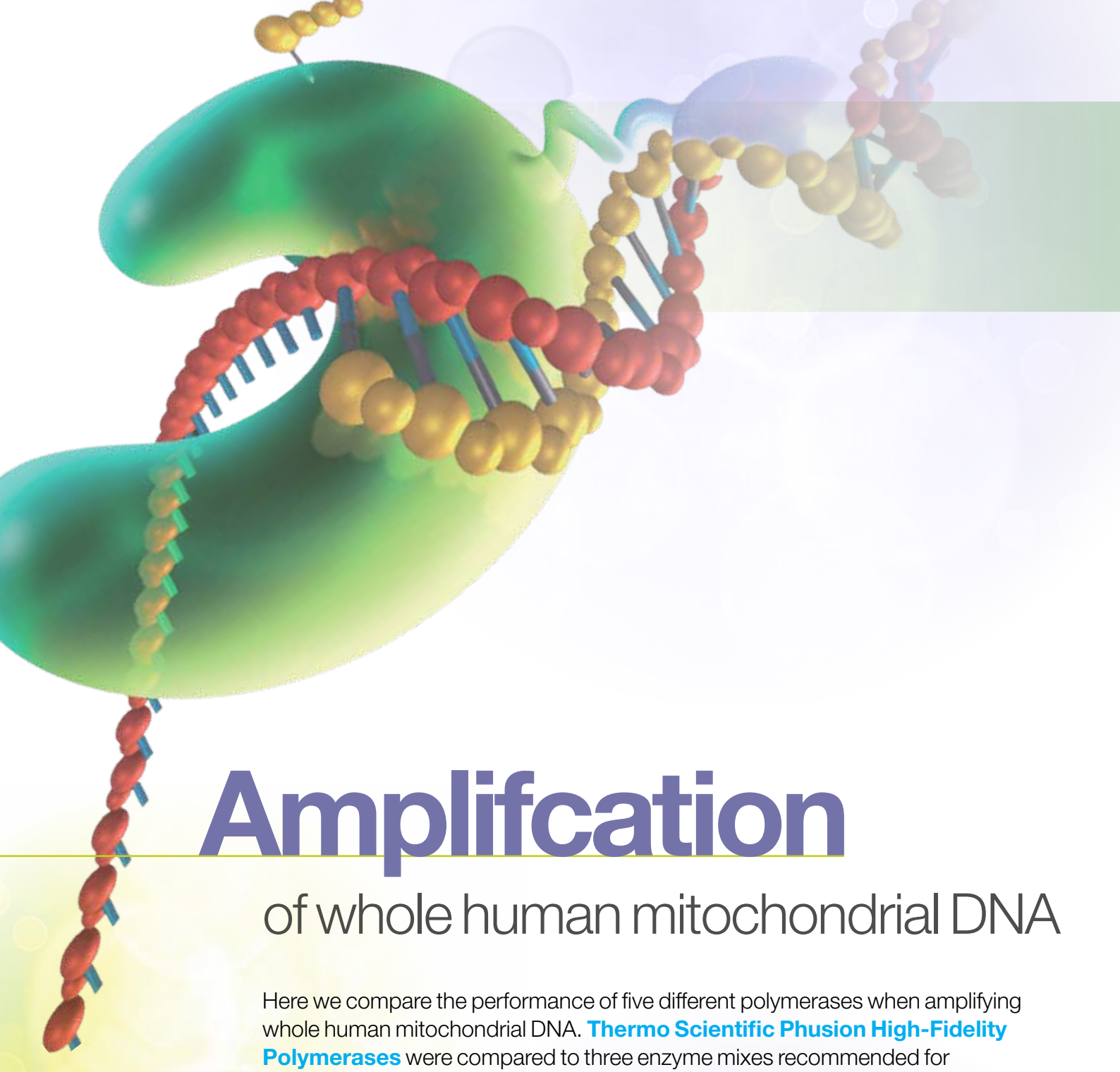


Amplifcation

of whole human mitochondrial DNA

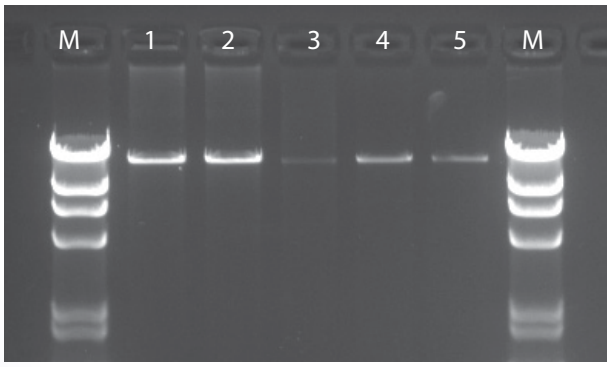
Here we compare the performance of five different polymerases when amplifying whole human mitochondrial DNA. **Thermo Scientific Phusion High-Fidelity Polymerases** were compared to three enzyme mixes recommended for amplification of long templates by the manufacturers. Phusion High-Fidelity DNA Polymerases performed the amplification in less time and with fewer units of enzyme than the other polymerases. Reliable amplification of human mitochondrial DNA can be accomplished more quickly and cost-effectively with Phusion High-Fidelity DNA Polymerases.

Introduction

Human mitochondrial DNA is a 16.6 kb circular double-stranded molecule. Mutations (deletions, duplications and point mutations) in the mitochondrial genome leading to mitochondrial dysfunction are increasingly recognised as a contributor to a wide range of human diseases. Mitochondrial dysfunction is involved in diseases such as diabetes, cancer, heart diseases and migraine. In addition, neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease are associated with mitochondrial dysfunction. Amplifying the whole mitochondrial genome using PCR has been found to be an efficient method for detecting mitochondrial DNA deletions involved in human diseases[1]. Amplifying the whole mitochondrial genome is useful for detecting relatively large mitochondrial deletions; for other mutations (e.g. point mutations), shorter target fragments are usually amplified.

Materials and Methods

Phusion High-Fidelity DNA Polymerases consist of a Pyrococcus-like enzyme with a double-stranded DNA-binding domain, which gives the fusion polymerase high processivity and fidelity. The performance of Phusion High-Fidelity DNA Polymerases and three polymerase mixes from two other vendors were compared by amplifying the whole human mitochondrial DNA. Total DNA isolated from human blood was used as a template. The amplified product was 16.5 kb.



Polymerase	Amount	Cycling time
1. Thermo Scientific Phusion High- Fidelity DNA Polymerase	1 U	4 h 48 min
2. Phusion® Hot Start High-Fidelity DNA Polymerase	1 U	4 h 48 min
3. Enzyme mixture from supplier R	2.6 U	7 h 40 min
4. Enzyme mixture from supplier T	2.5 U	7 h 49 min
5. Enzyme mixture from supplier T, hot start version	2.5 U	7 h 49 min

The primers used for the amplification anneal to mtDNA at the following positions: forward 10-40; reverse 16,494 –16,463[1]. All reactions were conducted using conditions recommended by the manufacturers. The amounts of enzymes in units and total cycling times are shown in Figure 1. The reactions were set up on ice and run on a thermal cycler.

Conclusions

The performance of five different polymerases was compared when amplifying whole human mitochondrial DNA. Phusion DNA Polymerases completed the entire PCR reaction in less than 5 hours, while the other polymerases required almost 8 hours. Compared to the three other polymerases tested, which are recommended by the manufacturers for amplification of long genomic targets, Phusion DNA Polymerases provided high yields with lower enzyme amounts. An important advantage of Phusion DNA Polymerases are their extremely low error rate. It is 50-fold lower than that of Taq polymerase. Two different buffers are provided with Phusion DNA Polymerases: Phusion HF Buffer (error rate 4.4 x 10^{-7}) and Phusion GC Buffer (error rate 9.5 x 10^{-7}). HF Buffer should be used as the default buffer for high fidelity amplification. However, GC Buffer can improve the performance on some difficult or long templates. Due to their high accuracy, Phusion DNA Polymerases can reliably be used for studying mitochondrial DNA point mutations. In conclusion, Phusion DNA Polymerases perform accurate and fast amplification of mitochondrial DNA.

The conditions for Phusion High-Fidelity DNA Polymerase and Phusion Hot Start DNA Polymerase were as follows:

- 50 ng template • 0.5 µM primers
- 200 µM dNTPs • 1 x GC buffer
- 1 U enzyme Total volume 50 µL

The cycling conditions for Phusion High-Fidelity DNA Polymerases were as follows:

Temperature	Time	Number of cycles
98 °C	30 s	1
98 °C	10 s	30 cycles
72 °C	8 min 15 s	
"72 °C 4 °C"	"10 min hold"	1

Acknowledgements
We thank Docent Anu Wartiovaara, Research programme of Neurosciences, University of Helsinki, Finland for the primer sequences.

Reference
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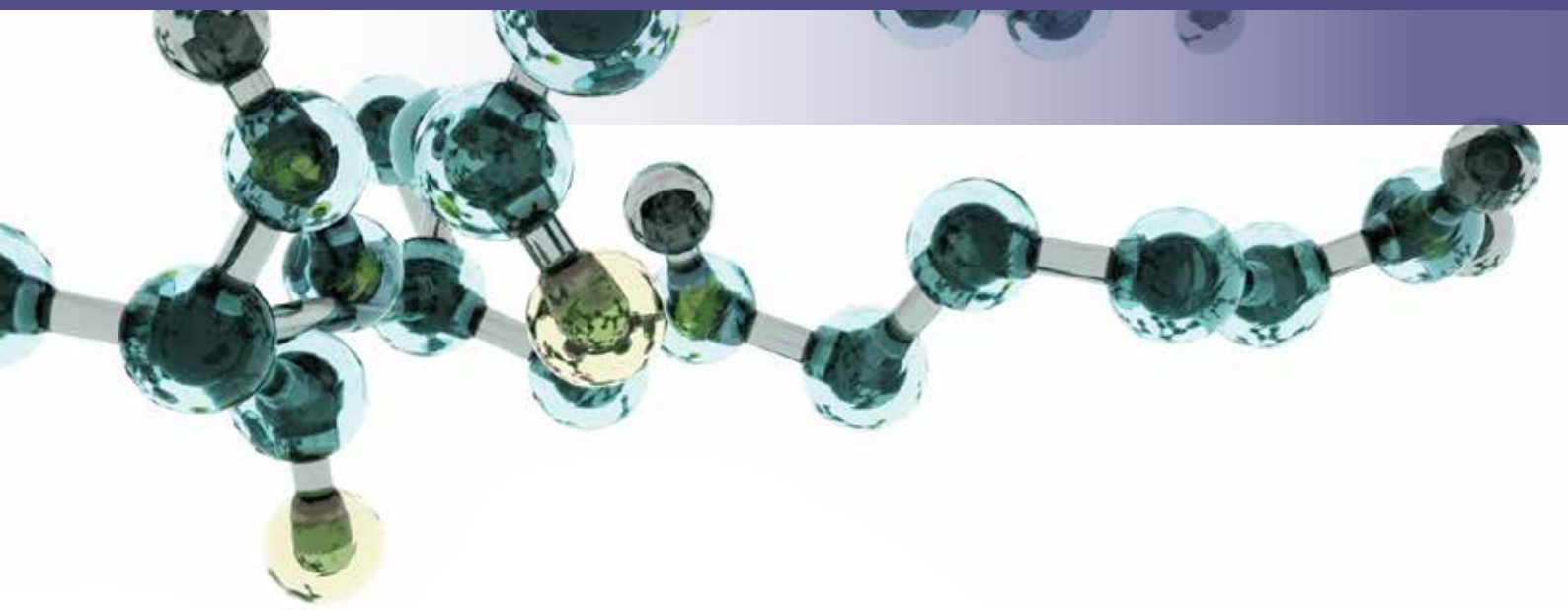

Iodoacetyl tandem mass tags

for cysteine peptide modification, enrichment & quantitation

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Overview

Purpose:
To develop an iodoacetyl Tandem Mass Tag (iodoTMT) reagent for irreversible cysteine peptide labeling, enrichment and multiplexed quantitation.

Methods:
Reduced sulfhydryls of protein cysteines were labelled with iodoTMTzero and/or iodoTMTsixplex reagents. Labelled peptides were enriched using an immobilised anti-TMT antibody resin before mass spectrometry (MS) analysis.

Results:
We developed an iodoTMT reagent set to perform duplex isotopic or sixplex isobaric mass spectrometry (MS) quantitation of cysteine-containing peptides. IodoTMT reagents showed efficient and specific labeling of peptide cysteine residues with reactivity similar to iodoacetamide. Using an anti-TMT antibody, we characterised the immunoaffinity enrichment of peptides labelled with iodoTMT reagents from complex protein cell lysates and for detection of S-nitrosylated cysteines.

Introduction

Thermo Scientific Tandem Mass Tag (TMT) Reagents enable concurrent identification and multiplexed quantitation of proteins in different samples using tandem mass spectrometry.[1] Previously, we described a cysteine-reactive, isobaric Tandem Mass Tag (cysTMT™) reagent that utilised a dithiopyridine reactive group to selectively label cysteine sulfhydryls. Although this labeling chemistry is highly specific and efficient, it results in a reversible di-sulfide linkage between the peptide and isobaric tag. Here, we report the development of an irreversible, cysteine-reactive TMT™ reagent containing an iodoacetyl reactive group (iodoTMT™). Due to the irreversible labeling of the iodoTMT reagent, it can be used for quantifying cysteine modifications such as S-nitrosylation, oxidation and disulfide bridges.

Sample Preparation

Preparation of iodoTMT-labelled proteins: Proteins and/or cell lysates were solubilised at 2 mg/mL in 50 mM HEPES pH 8.0, 0.1% SDS and reduced with 5 mM TCEP for 1 hour at 50°C. Reduced proteins were labelled with 5-10 mM iodoTMT reagent (~10 molar excess) for 1 hr at 37°C protected from light. Excess iodoTMT reagent was removed by acetone precipitation of samples at -20°C for 4-20 hrs.
Proteins were enzymatically digested at 37°C for 4 hrs and desalted before liquid chromatography (LC)-MS/MS analysis or enrichment.

Enrichment of iodoTMT-labelled peptides: Labelled peptides (25–100 µg) were resuspended in TBS at 0.5 µg/µL and incubated with an immobilised anti-TMT antibody resin (20–100 µL) overnight with end-over-end shaking at 4°C. After collection of the unbound sample, the resin was washed 4X with 4 M Urea/TBS, 4X with TBS, and 4X with water. Peptides were eluted 3X with 50% acetonitrile/0.4% TFA, frozen, and then dried under vacuum before LC-MS/MS analysis.

Selective labeling of S-nitrosylated proteins: Proteins and cell lysates were solubilised at 2 mg/mL in modified HENS buffer (100 mM HEPES pH 8.0, 1 mM EDTA, 0.1 mM neocuporine, 1% SDS). Free sulfhydryls were blocked with methyl methane thiosufate (MMTS) for 20 min at room temperature and desalted to remove excess blocking reagent. S-nitrosylated cysteine sulfhydryls were selectively labelled using 0.4mMiodoTMT reagent in the presence of 20 mM sodium ascorbate. Excess iodoTMT reagent was removed by acetone precipitation of samples at -20°C for 4-20 hrs. Unlabelled cysteines were reduced and alkylated with 20 mM iodoacetamide. Proteins were enzymatically digested at 37°C for 4 hrs and desalted before LC-MS/MS analysis or enrichment.

LC-MS/MSAnalysis: A nanoflow high-pressure liquid chromatography system with a Thermo Scientific PepMap C18 column (75 µm ID x 20 cm) was used to separate peptides using a 5%-40% gradient (A: water, 0.1% formic acid; B: acetonitrile, 0.1% formic acid) at 300 nL/min over 60 min. A Thermo Scientific LTQ Orbitrap XL ETD linear ion trap mass spectrometer was used to detect peptides using a top-3 CID, -3 HCD experiment for peptide identification and reporter ion quantitation.

Data Analysis: MS spectra were searched using Thermo Scientific Proteome Discoverer software v1.3

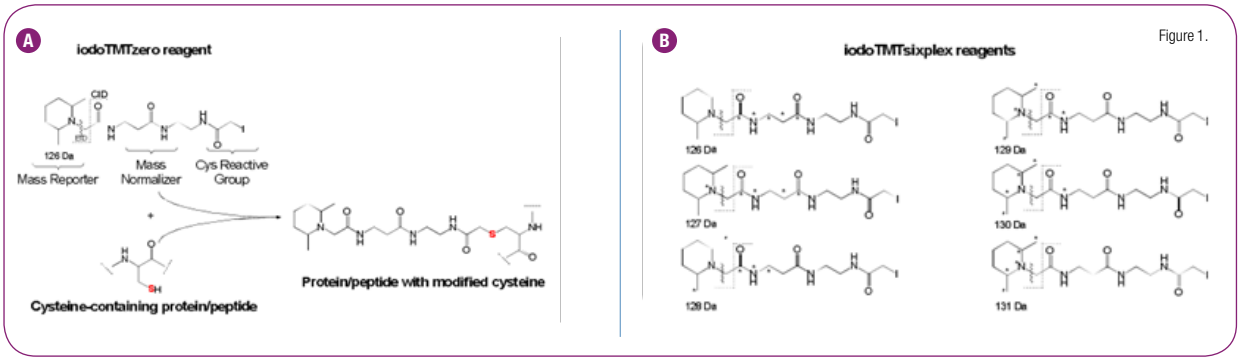

Figure 1.

FIGURE 1. iodoTMT reagents and labeling reaction.
(A) Mechanism of iodoTMT reagent reaction with cysteine containing proteins or peptides.
(B) Structure of iodoTMTsixplex reagents for cysteine labeling, enrichment, and isobaric MS quantitation

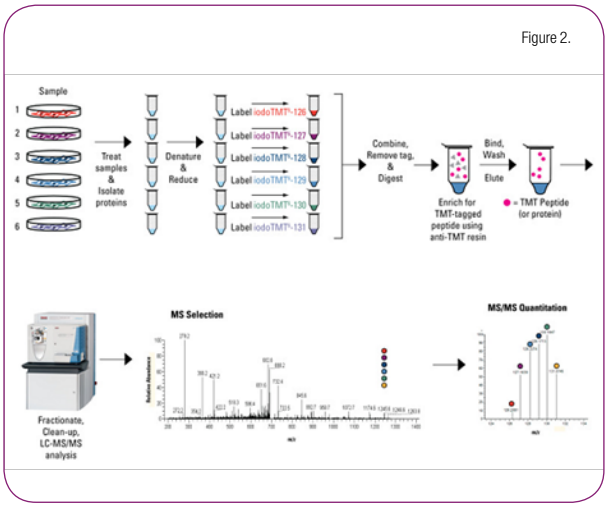

Figure 2.

FIGURE 2. Schematic of iodoTMTsixplex reagent workflow. Six different sample conditions can be prepared for iodoTMT reagent labeling. Labelled proteins are combined before iodoTMT peptide enrichment using immobilised anti-TMT antibody resin and subsequent LC-MS/MS analysis of isobaric reporter ions.

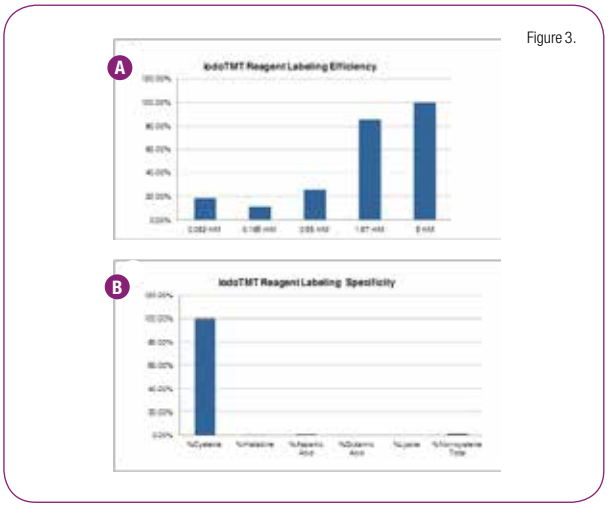

Figure 3.

FIGURE 3. iodoTMT reagent labeling specificity and efficiency. A) Reduced BSA (100 µg) was labelled with increasing concentrations of iodoTMT reagent. IodoTMT reagent labeling efficiency was determined by peptide signal (XIC) of modified of cysteines compared to total cysteine-containing peptides signal. B) Reduced BSA (100 µg) was labelled with 10 mM iodoTMT reagent. IodoTMT reagent labeling specificity was determined by comparing modified peptide signal to total peptide signal for different amino acids. Labeling specificity and efficiency were also assessed by peptide spectral counting which gave similar results (data not shown).

Figure 4.

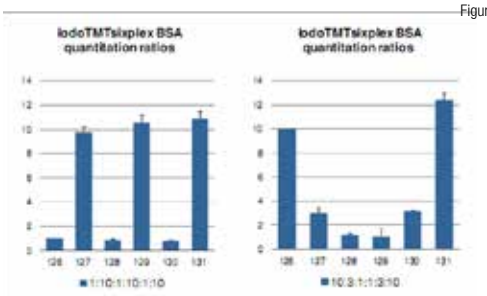

FIGURE 4. iodoTMTsixplex reagent relative quantitation of BSA peptides. Reduced BSA (100 µg) was labelled with 10 mM iodoTMTsixplex reagent and combined in fixed ratios (126:127:128:129:130:131 = 10:3:1:1:3:10 & 1:10:1:10:1:10) before protein digestion. Graph of peptide relative quantitation for all quant.

Figure 5.

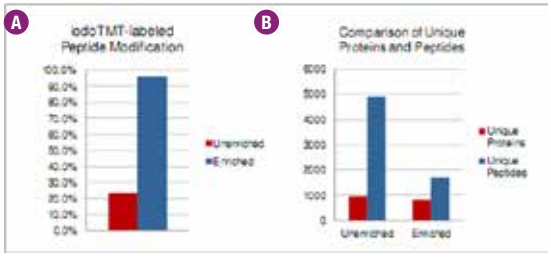

FIGURE 5. Anti-TMT enrichment of iodoTMT-labelled peptides. A) Percent of iodoTMT-labelled peptide modifications identified before and after enrichment. B) Comparison of unique proteins identified from unique peptides from A549 cell lysates before and after anti-TMT antibody resin enrichment.

Figure 6.

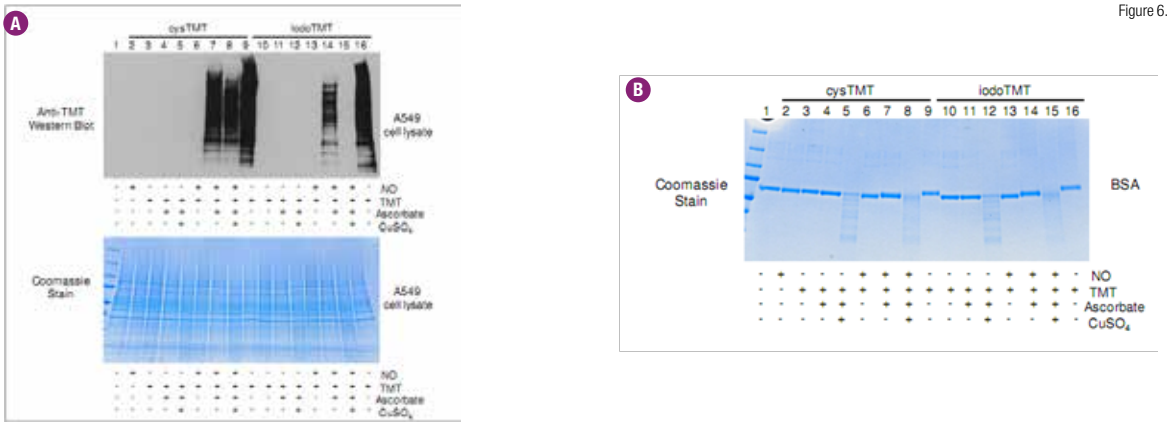

FIGURE 6. cysTMT and iodoTMT reagent labeling of S-nitrosylated proteins. A549 cell lysates (A) and BSA (B) were blocked with MMTS (lanes 1-8, 10-15) or untreated (lanes 9, 16) before 500 mM nitro-glutathione (NO) treatment and TMT reagent labeling in the presence or absence of ascorbate and copper sulphate (CuSO4). Proteins were separated by SDS-PAGE and analysed by anti-TMT antibody Western blotting or Coomassie stain.

Results

We have developed and used an iodoacetyl TMT reagent (iodoTMT) to irreversibly label sulfhydryls of cysteine-containing peptides for multiplex quantitation by LC-MS (Figure 1). Compared to the cysTMT reagent workflow, the iodoTMT reagent workflow is simpler since reducing agents are not removed from protein samples before labelling (Figure 2). The iodoTMT reagents showed efficient and specific labeling of peptide cysteine residues with reactivity similar to iodoacetamide (Figure 3A & 3B). IodoTMT reagents were also used for sixplex isobaric quantitation of cysteine-containing peptides (Figure 4). We also characterised an anti-TMT antibody developed against the reporter region of the TMT reagent for immuno-enrichment of iodoTMT-labelled peptides (Figure 5) and Western blot detection of iodoTMT-labelled proteins (Figure 6A).

We used the iodoTMT reagent as a probe for labeling S-nitrosylated cysteines in a modified S-nitro switch assay (Figure 6). IodoTMT reagents successfully labelled S-nitrosylated cysteines after selective reduction using ascorbate; however, labelling efficiency was less than cysTMT reagents. This result is consistent with different efficiencies of the sulfhyrdyl-reactive groups (dithiopryridine vs. iodoacetyl) of each reagent. In addition, we discovered that addition of 1mM copper sulfate to the switch reaction buffer inhibited iodoTMT reagent labeling but not cysTMT reagent labeling.

Addition of copper sulfate is thought to facilitate S-NO bond reduction during the labeling reaction; however, in the presence of ascorbate, Cu^{2+} is readily reduced to Cu^{1+}, which can generate free radicals.[2] The free radicals generated resulted in protein degradation (Figure 6B: lanes 5, 8, 12 and 15) and possible loss of iodine from the iodoacetyl reactive group.

Overall, using the combination of iodoTMT labeling with anti-TMT enrichment has several advantages over previously described cysteine-reactive workflows[3,4] for labeling, enrichment and quantitation of cysteine-containing peptides and cysteine modifications such as S-nitrosylation

Conclusions

- Iodoacetyl Tandem Mass Tags (iodoTMT) are novel reagents for specific irreversible labeling of cysteine residues pre- or post-digestion.
- iodoTMT reagents can be used as either isotopic pairs or as an isobaric set for MS- or MS/MS-based multiplexed quantitation.
- An antibody to the TMT reagent reporter region allows specific detection, capture, and enrichment of iodoTMT-labelled proteins and peptides.
- iodoTMT reagents can be used for detection of cysteine modifications such as S-nitrosylation.

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Acknowledgments

The authors would like to thank Dr. Jennifer Van Eyk and Dr. Christopher Murray (Johns Hopkins) for sharing preliminary data on S-nitrosylation switch assay protein labeling conditions.

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